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News

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. . . *THROUGH CHEMISTRY*

PURPLE AND BROWN BLOTCH OF ONIONS [1]

R. L. Skiles [2]

Purple blotch has become one of the limiting factors in onion (*Allium cepa* L. var. Sweet Spanish) production in the Arkansas Valley of Colorado. Estimated losses in field and storage often have ranged from 30 to 50 per cent and have sometimes been as high as 100 per cent. Bulb rot in storage, where the greatest losses occur, is preceded by purple blotches on the foliage of the growing plants in the field. The causal organism attacks the leaves or leaf sheaths, grows into the neck of the onion and finally into the bulb. Symptoms of bulb rot often escape observation until after storage periods of 1 to 5 months.

In 1948 and 1949 it was noticed that field symptoms of the disease were different from those which have been described for purple blotch caused by *Alternaria porri* (Ellis) Ciferri (1, 5). There was a high incidence of brown blotch as well as purple blotch in nearly all onion fields; the former symptom usually preceded the latter in seasonal appearance. In addition, many lesions, when formed, were part purple and part brown, and remained so throughout the growing season. After the initial appearance of brown blotch and purple blotch, new lesions of either one or both colors were continually formed until harvest.

The following study was made to determine whether the leaf blotches of different colors were within the range of symptom variation of *A. porri*, or if more than 1 fungus was involved in a complex of diseases with overlapping symptoms.

REVIEW OF LITERATURE.—The binomial combination *Alternaria porri*, for the purple-blotch fungus, was first used by Ciferri (2) in 1930; previous to that time it was described as *Macrosporium porri* Ellis. The fungus was first reported in the United States by Cooke and Ellis (3), in 1879, on leek (*Allium porrum* L.). Nolla (6) described *Alternaria allii* on onions; he stated that the purple may fade to buff in the old lesions, remaining purple only in the center. Angell (1), however, held the opinion that *A. allii* and *M. porri* were identical except for minor variations which did not warrant species distincton. He also showed that *M. porri* could cause bulb rot as well as purple foliage lesions and that the fungus never formed chains of more than 2 spores.

[1] Accepted for publication March 30, 1953.

Portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree, Colorado A&M College. Published with the approval of the Director as Scientific Series Paper 393, Colorado Agricultural Experiment Station.

This investigation was made possible by a grant from the Associated Growers and Shippers of Colorado.

The author wishes to express his gratitude to Dr. L. W. Durrell, Head of the Department of Botany and Plant Pathology, Colorado A&M College, under whose guidance the work was done.

[2] Present address: Oficina de Investigaciones Especiales, Facultad de Agronomia, Medellin, Colombia, S. A.

Brown blotch has been ascribed to infection by *Stemphylium botryosum* Wallroth (= *Macrosporium parasiticum* Thuemen). Teodoro (8) demonstrated that this fungus was a pathogen on onion foliage. However, Machacek (4) reported later that it would not cause bulb rot and that it was a secondary leaf parasite following injury by downy mildew, *Peronospora destructor* (Berk.) Casp. While working with isolates of *M. parasiticum* obtained from onion leaves, Young (9) obtained onion bulb-membrane infection but was not able to reinfect leaves. He also used isolates of *Alternaria tenuis* obtained from wheat seed and *Datura tatula* leaf spot and found that they would not infect onion bulb-membranes or leaves.

MATERIALS AND METHODS.—Isolations were made from diseased material obtained from onion fields and storage houses in the Arkansas Valley and from the Colorado A&M College horticultural gardens. Sixteen isolates of *Alternaria* were selected for use in these studies. Cultures originated from single hyphal tips and were sub-cultured continuously on potato-dextrose agar. The code numbers and sources of the cultures are as follows:

PN-1 —spores scraped from the edge of a brown leaf lesion,
PN-2 —isolation from a bulb with black rot preceded by a red line of fungus advance,
PM-6 —isolation from a purple leaf lesion,
PM-12 —isolation from a light brown leaf lesion,
PM-20 —isolation from a purple leaf lesion,
PM-25 —isolation from a bulb with slight blackening, the bulb appeared to be in a brown, water-soaked stage or rot,
PM-26 —isolation from a brown leaf lesion,
PII —spores scraped from a purple leaf lesion,
PIV —spores scraped from a leaf lesion that was part purple and part brown,
PV —spores scraped from a faint purple leaf lesion,
PVI —spores scraped from a very large, purple leaf lesion,
PVII —spores scraped from a dark purple seed-stalk lesion,
PVIII —spores scraped from a large, brown leaf lesion with a small purple center,
PIX —spores scraped from the distal portion of a brown leaf lesion where it tapered into a long chlorotic streak,
PXII —spores scraped from a purple leaf lesion,
PXV —isolation of white, cottony, fungous growth on a purple leaf lesion.

When the experiments were about half completed culture PXII was lost because it failed to maintain growth after successive transfers on agar.

Two strains of the Yellow Sweet Spanish variety were used for bulb inoculations: Colorado 6 and a Ferry-Morse selection of Utah. These bulbs were grown in the field, consequently they were carefully examined to determine that no incipient neck-, bulb-,

TABLE 1.—*Species classification of Alternaria cultures*

A. porri	A. tenuis	A. tenuissima
PN-1	PV	PM-12
PN-2	PXV	PM-20
PII		PM-25
PIV		PM-26
PVI		PIX
PVII		
PVIII		
PXII		

or base (stem) rot was present before they were inoculated. Leaf inoculations were made in the field on mature Colorado 6 plants. A selected leaf was washed with distilled water, a bit of agar and mycelium was placed on it; it was then covered with a transparent paper bag. After 5 days the bags were removed and 2 days later disease readings were made. Bulb inoculations were made as follows: a small area on the outside of the bulb was washed with alcohol, a plug was removed with a sterile cork-borer, a piece of agar and mycelium was placed in the bottom of the cavity, the plug was then replaced and sealed with transparent tape. The bulbs were stored for 2 months at approximately 20° C.; this temperature was higher than optimum for bulb storage but it was favorable for the development of bulb rot.

Check bulbs and leaves were included in all the experiments. They were treated in the same manner as the inoculated material with the exception that no mycelium was placed on the plant part.

CLASSIFICATION OF ALTERNARIA CULTURES.—The 16 cultures were separated into 3 species: *Alternaria porri*, *A. tenuis auct. sensu lato*, and *A. tenuissima* (Fr.) Wiltshire (Table 1). The species descriptions given by Neergaard (5) were used for the identifications.

The *A. porri* cultures sporulated only on corn meal agar after use of a modification of Rands' (7) method for inducing sporulation in which the mycelial mat was macerated and exposed to direct sunlight for 4 days. PM-6 is not listed in Table 1 since this culture never sporulated on an artificial medium or on onion leaves in the greenhouse. It was later classified as *A. porri* because of its pathogenicity on bulbs. Also, PM-25 never sporulated on an artificial medium, but did so on onion leaves in the greenhouse.

PATHOGENICITY ON BULBS.—Respective lots of 10 bulbs were inoculated with each fungus. The degree of pathogenicity on each bulb was rated on the following basis: very pathogenic, moderately pathogenic, slightly pathogenic, very slightly pathogenic, or nonpathogenic (VP, MP, SP, VSP, or NP). The limits of each class were determined by the extent of fungous growth in the bulb as manifested by brown or black rot. Results of the bulb inoculations are in Table 2.

The *A. porri* cultures were, in general, considerably more pathogenic than those of the other 2 species. Within *A. tenuis*, PXV was slightly to moderately pathogenic and PV was very slightly pathogenic; nevertheless, either culture could greatly reduce the marketability of the infected bulbs. Rot symptoms caused by pathogenic cultures of *A. tenuis* and *A. tenuissima* were indistinguishable from the symptoms caused by *A. porri*.

Since PM-6 never sporulated it could not be classified as a species of *Alternaria*. However, it was placed

TABLE 2.—*Results of inoculation of 2 strains of onion bulbs with different cultures of A. porri, A. tenuis, and A. tenuissima*

Fungus Species and culture	Colorado 6					Ferry-Morse				
	VP	MP	SP	VSP	NP	VP	MP	SP	VSP	NP
A. porri										
PN-1	10					10				
PN-2	10					9	1			
PM-6	10					10				
PII	10					10				
PIV	10					10				
PVI	10					9	1			
PVII	10					10				
PVIII	10					10				
PXII	*					10				
A. tenuis										
PV				10						10
PXV	6	2			2		2			8
A. tenuissima										
PM-12	1				9					10
PM-20			2	1	7	2	1			7
PM-25					10				2	8
PM-26	1				9		1			9
PIX		1	1		8	1	1			8
Check	0	0	0	0	0	0	0	0	0	0

* No bulbs inoculated

TABLE 3.—*Results of foliage inoculations with different cultures of A. porri, A. tenuis, and A. tenuissima*

Species and culture	Uninjured leaves		Injured leaves					
	Number lesions	Number purple lesions[a]	Number lesions					Number purple lesions[a]
			VP	MP	SP	VSP	NP	
A. porri								
PN-1	1	0	3	2				1
PN-2	5	1	5					5
PM-6	5	2	5					2
PII	4	0	5					2
PIV	4	0	5					2
PVI	1	0	5					2
PVII	1	0	5					2
PVIII	4	3	5					1
PXII	1	0	5					4
A. tenuis								
PV	0	0	1	3	1			0
PXV	0	0	5					2
A. tenuissima								
PM-12	1	0			1		4	0
PM-20	0	0	1	2	2			1
PM-25	0	0	1	2	2			0
PM-26	3	0		2	2	1		0
PIX	0	0	2	1	1		1	0
Check	0	0	0	0	0	0	0	0

[a]Lesion entirely or partly purple, other lesions were brown.

in the *A. porri* group because it was more pathogenic on bulbs of both onion strains than any of the cultures in the other 2 species (Table 2).

PATHOGENICITY ON LEAVES.—Field-grown Colorado 6 onions were inoculated with each culture on 1 mechanically injured leaf on each of 5 plants and 1 uninjured leaf on each of 5 different plants. The inoculated leaves were all approximately the same age. The degree of pathogenicity of each culture was rated on the following basis: very pathogenic, moderately pathogenic, slightly pathogenic, very slightly pathogenic, or nonpathogenic (VP, MP, SP, VSP, or NP) according to the size of the leaf lesions. This classification was employed only on leaves that had previously been injured. It was believed that more uniform conditions were maintained on these leaves than on the uninjured ones. As shown in Table 3, only *A. porri* caused purple lesions on uninjured foliage, and then only rarely. On injured foliage, all 3 fungi gave rise to purple lesions; however, *A. porri* formed more of them than did the other 2 species. Conversely, brown lesions or vari-colored lesions resulted from infection by all 3 *Alternaria* species. The pathogenicity of each species was greater on injured than on uninjured leaves and *A. porri* was generally the most pathogenic of the 3.

DISCUSSION.—The name "purple blotch," as formerly used in the literature and by the onion growers in the Arkansas Valley, connoted infection by *Alternaria porri*. It was commonly used to describe bulb rotting in storage as well as leaf blotching in the field. The term "brown blotch" has been associated with infection by *Stemphylium botryosum*. The latter fungus was isolated occasionally from purple and brown leaf lesions but was eliminated from these studies because it was not consistently associated with the disease. It has been shown here that both the purple and the brown leaf lesions may result from infection by *A. porri*, *A. tenuis*, or *A. tenuissima*. Therefore, it appears that the names "purple blotch" and "brown blotch" are undesirable because neither is indicative of a disease caused by one organism. Instead, the name "Alternaria blotch" is proposed to describe the disease complex. It is understood from the new name that 3 species of *Alternaria* cause the disease, and that the leaf and bulb pathogenicities are variable within and between the species.

Results indicate that foliage injury is almost a prerequisite to infection by *A. tenuis* or *A. tenuissima*. The action of such agents as thrips (*Thrips tabaci*), hail, or wind-blown soil in causing leaf injury is quite common in the field. Therefore, it is possible that these 2 species may do considerable damage, especially by top-killing.

The action of *A. porri* in causing bulb rot was shown to be much faster than that of *A. tenuis* or *A. tenuissima*. Under conditions of rapid movement of bulbs from field to market it is likely that the effects of the latter 2 species may be negligible. However, bulbs are frequently stored for 1 to 4 months before shipment. Then the losses from *A. porri* may be greater in number but less in monetary value than the losses from *A. tenuis* and *A. tenuissima*. When bulbs are removed from storage they are graded before shipment and severely rotted ones are discarded. Any rots that cannot be detected by sponginess or distortion of the bulbs are passed by the graders and moved to the market. As a result, the slower type of rot character-

istic of *A. tenuis* and *A. tenuissima* infection may be more costly because shipping expenses are added to the lost value of the bulbs.

SUMMARY

Symptoms of purple blotch on onion foliage were found to intergrade often with those of brown blotch. The diseases have been associated previously with 2 different fungi.

Alternaria porri, A. tenuis, and *A. tenuissima* were isolated from rotted bulbs and from leaves with purple, brown, and mixed blotches.

Leaves of 1 strain of Yellow Sweet Spanish onion and bulbs of 2 strains were inoculated with several isolates within each fungous species.

A. porri was more pathogenic than *A. tenuis* or *A. tenuissima* on bulbs. Rots caused by the latter 2 species were sufficient to reduce the marketability of the bulbs.

On uninjured and mechanically injured foliage, *A. porri* was more pathogenic than *A. tenuis* or *A. tenuissima.*

The cultures within each fungous species were more pathogenic on injured than on uninjured leaves.

On uninjured leaves only *A. porri* formed purple lesions, while on injured foliage all 3 species formed them. In the latter case *A. porri* formed a greater percentage of purple blotches than did *A. tenuis* or *A. tenuissima.*

Brown blotches resulted from infection by all cultures of each fungus.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
COLORADO A&M COLLEGE
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SEROLOGICAL TESTS WITH SOME PHYTOPATHOGENIC SPECIES OF PSEUDOMONAS [1]

B. A. Friedman [2]

In view of the findings in this laboratory of the morphological and physiological similarity of a number of fluorescent cultures of *Pseudomonas* isolated from decayed potato tubers, witloof chicory, lettuce and escarole (6, 7), serological methods were tried in order to identify the isolates. For this purpose unknown isolates as well as 23 named cultures comprising 14 species of *Pseudomonas* were compared by means of agglutination tests with antisera prepared against *Ps. syringae, Ps. marginalis, Ps. intybi* and *Ps. fluorescens.* The present paper reports the results of the agglutination tests with the named cultures only.

METHODS AND MATERIALS.—Cultures were streaked on nutrient agar plates to determine their apparent purity, and morphological and physiological tests were made to determine their identity. Forty-eight to 72-hour old motile cultures of *Ps. syringae* PS20, *Ps. marginalis* 249, *Ps. intybi,* and *Ps. fluorescens* B10 were killed with 0.5 per cent formaldehyde and were used to prepare antisera by means of 6 weekly inoculations of rabbits. Macroscopic tube agglutination tests, employing antiserum dilutions ranging from 1:20 to 1:10240, were used to test the agglutination reactions of the 23 cultures studied. Standard procedures were followed for the preparation of antigens and antisera and for the performance of agglutination tests (10).

Tests were conducted with the following organisms: *Ps. aeruginosa* (Schroeter) Migula, No. 10145, *Ps. aptata* (Brown and Jamieson) Stevens, No. 10205, *Ps. coronafaciens* (Elliott) Stapp, No. 9005, *Ps. fluorescens* Migula, No. 9721, *Ps. syringae* van Hall, No. 7432 and *Ps. viridilivida* (Brown) Holland, No. 512 received from the American Type Culture Collection; *Ps. cichorii* (Swingle) Stapp, PC1, *Ps. syringae,* PS20, and *Ps. tomato* (Okabe) Bergey et al., PT7 from W. H. Burkholder; *Ps. marginalis* (Brown) Stevens, Nos. 136 and 249 from W. J. Dowson; *Ps. syringae,* B143 from W. H. English; *Ps. marginalis,* CIBP isolated by the author; *Ps. fluorescens,* B10 from W. C. Haynes; *Ps. fluorescens,* We, and No. 949 from M. Scherago; *Ps. fluorescens,* A.3.12 from R. Y. Stanier; *Ps. intybi* (Swingle) Stapp from C. Stapp; and *Ps. aceris* (Ark) Burkholder, PA1, *Ps. asplenii* (Ark and Tompkins) Savulescu, PA17, *Ps. lapsa* (Ark) Burkholder, PL2, *Ps. primulae* (Ark and Gardner) Starr and Burkholder, PP3 and *Ps. syringae* PS9 from M. P. Starr.

RESULTS.—The results of the agglutination tests

[1] Accepted for publication March 30, 1953.

[2] Plant pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

TABLE 1.—*Macroscopic tube agglutination reactions of some species of Pseudomonas* [a]

Test No.	Cultures tested	Antisera prepared in rabbits against antigens of: *Ps. syringae* PS20	*Ps. marginalis* 249	*Ps. fluorescens* B10	*Ps. intybi*
1.	*Ps. syringae* PS20	2560, 2560	0, 0	160, 80	80, 40
2.	*Ps. syringae* PS9	1280, 640	0, 0	80, 80	40, 40
3.	*Ps. syringae* 7432	0, 0	0, 0	0, 0	0, 0
4.	*Ps. syringae* B143	1280, 640	0, 0	0, 0	0, 0
5.	*Ps. marginalis* 249	0, 0	5120, 2560	0, 0	0, 0
6.	*Ps. marginalis* CIBP	0, 0	0, 0	0, 0	0, 0
7.	*Ps. marginalis* 136	0, 0	0, 0	0, 0	0, 0
8.	*Ps. fluorescens* B10	80, 160	0, 0	5120, 2560	0, 0
9.	*Ps. fluorescens* 9721	0, 0	0, 0	0, 0	0, 0
10.	*Ps. fluorescens* A.3.12	80, 160	0, 0	0, 0	0, 0
11.	*Ps. fluorescens* We	160, 40	0, 0	20, 40	40, 160
12.	*Ps. fluorescens* 949	0, 0	0, 0	0, 0	0, 0
13.	*Ps. intybi*	0, 0	0, 0	0, 0	5120, 1280
14.	*Ps. cichorii* PC1	0, 0	0, 0	0, 0	0, 0
15.	*Ps. aptata* 10205	160, 40	0, 0	0, 0	20, 20
16.	*Ps. coronafaciens* 9005	40, 40	0, 0	0, 0	0, 0
17.	*Ps. tomato* PT7	80, 40	0, 0	80, 20	0, 0
18.	*Ps. viridilivida* 512	0, 0	0, 0	0, 0	0, 0
19.	*Ps. aceris* PA1	80, 80	0, 0	40, 80	20, 20
20.	*Ps. primulae* PP3	0, 0	0, 0	0, 0	0, 0
21.	*Ps. asplenii* PA17	0, 0	0, 0	0, 0	0, 0
22.	*Ps. lapsa* PL2	2560, 1280	0, 0	0, 0	0, 0
23.	*Ps. aeruginosa* 10145	0, 0	320, 320	0, 0	0, 0

[a] Each figure represents the reciprocal of the highest dilution, i.e., "titer," showing a two-plus tube agglutination reaction; each figure represents one replication.

with 23 cultures against 4 antisera are given in Table 1. The antigens used to prepare the antisera reacted to a high titer with the homologous antisera (Tests 1, 5, 8 and 13). In addition, *Ps. syringae* PS9 and B143 and *Ps. lapsa* reacted to a high titer against *Ps. syringae* PS20 antiserum (Tests 2, 4 and 22). Low titer heterologous cross reactions were observed with 10 cultures, namely *Ps. syringae* PS20 and PS9, *Ps. fluorescens* B10, A.3.12, and We, *Ps. aptata*, *Ps. coronafaciens*, *Ps. tomato*, *Ps. aceris*, and *Ps. aeruginosa* (Tests 1, 2, 8, 10, 11, 15, 16, 17, 19 and 23). Nine cultures showed no agglutination reactions with any of the antisera (Tests 3, 6, 7, 9, 12, 14, 18, 20 and 21).

Because of the high titer reaction of *Ps. lapsa* in *Ps. syringae* antiserum (Test 22), the physiological reactions of these 2 organisms were compared. It was found that both organisms form fluorescein but no pyocyanin in asparagine broth, reduce nitrates slightly, fail to form indol, liquefy gelatin, form acid but no gas (in brom cresol purple synthetic broth) from dextrose, sucrose, and glycerol but no acid from maltose and lactose, grow with an alkaline reaction in lactic acid, citric acid, and succinic acid broths, and fail to grow or form alkali in sodium tartrate broth.

DISCUSSION.—Serological reactions with a high titer are considered to indicate a specific relationship (11, 15, 20). Aside from the 4 cultures used to prepare the antisera, only 3 of the remaining 19 cultures were agglutinated to high titer. One of these 3 cultures, namely *Ps. syringae* PS9, was isolated originally by W. H. Burkholder from lilac as was *Ps. syringae* PS20, which was used to prepare the antiserum. *Ps. syringae* B143, which also reacted at a high dilution with *Ps. syringae* PS20 antiserum, was isolated by E. E. Wilson from a plum canker.

Ps. lapsa, the third culture observed to agglutinate to a high titer with *Ps. syringae* antiserum, was isolated by P. A. Ark from diseased field corn. These 2 species have been reported to infect corn; *Ps. syringae* has not been reported to be pathogenic to sugar cane while *Ps. lapsa* has (1, 4). The serological and physiological results suggest that *Ps. lapsa* and *Ps. syringae* may be identical, but it should be emphasized that only 1 culture of *Ps. lapsa* was available and there was no antiserum against it. Consequently it was not possible to test by reciprocal agglutination absorption the identity of the 2 cultures. In addition, cross inoculation of field corn and sugar cane with freshly isolated cultures of the 2 organisms would be desirable to compare their relationship further.

The heterologous cross reactions found with *Ps. syringae* PS20 and PS9, *Ps. fluorescens* B10, A.3.12, and We, *Ps. aptata*, *Ps. coronafaciens*, *Ps. tomato*, *Ps. aceris*, and *Ps. aeruginosa* indicate the presence of common antigenic substances in these organisms and the bacteria used for the preparation of the corresponding antisera. Such low titer reactions are generally considered to mean the lack of serological specificity (11, 15, 20). Similar heterologous reactions have been reported for *Ps. syringae* (16, 19), *Ps. aptata* (18), *Ps. coronafaciens* (18), *Ps. fluorescens* (13, 16, 19) and *Ps. aeruginosa* (5, 9, 18).

The 5 cultures (*Ps. syringae* 7432, *Ps. marginalis* 136, CIBP, and *Ps. fluorescens* 9721, 949) which

showed no agglutination in the corresponding antisera may be considered distinct serologically and may represent either different types or strains, culture phases, or mutants of their parent cultures (2, 15, 20) or may have been named incorrectly in the first place. The remaining 4 cultures (*Ps. cichorii, Ps. viridilivida, Ps. primulae* and *Ps. asplenii*) may be considered to be distinct serologically from the 4 cultures used to produce the antisera. It should be noted that *Ps. primulae* has been reported to cross-agglutinate to high titer with *Ps. syringae* and *Ps. fluorescens* (16).

The agglutination of *Ps. aeruginosa* by *Ps. marginalis* 249 antiserum at a titer of 320, which is low but slightly higher than most cross reactions found in these tests, is interesting in view of the fact that *Ps. marginalis* has been reported to be identical with *Ps. aeruginosa* (5, 12). It has been shown, however, that M. M. Mehta's culture of *Ps. marginalis* produced pyocyanin while a culture received from E. F. Smith did not (18). In addition, a lettuce organism which was reported as probably *Ps. aeruginosa* (14) was not identical with a culture of *Ps. marginalis* received from N. A. Brown. The 3 cultures of *Ps. marginalis* used in the present tests produced fluorescein, but no pyocyanin even when examined in the dark by ultra-violet light, although apocyanogenic strains of *Ps. aeruginosa* are known (13). At present, however, strains of *Ps. marginalis* that produce pyocyanin (in addition to fluorescein) are considered to be *Ps. aeruginosa*, while strains which produce fluorescein only are called *Ps. marginalis* (3).

The number of cultures used in these tests was small and the results are interpreted to mean only that in some cases, as with *Ps. syringae*, serological tests may be helpful in the identification of cultures (8, 17) while in other cases, as *Ps. marginalis* and *Ps. fluorescens*, agglutination reactions may be less helpful because of the serological heterogeneity among various species of *Pseudomonas* (5, 9, 13, 18). Further serological analysis of the species of *Pseudomonas* may prove worthwhile, as it has been concluded that serological reactions, in addition to morphological and biochemical methods, are essential for the identification and classification of members of the genus *Pseudomonas* (13).

SUMMARY AND CONCLUSIONS

A culture of *Ps. lapsa* and 2 of 3 cultures of *Ps. syringae* apparently reacted specifically with *Ps. syringae* antiserum.

Ten cultures representing 7 species of *Pseudomonas* showed heterologous cross reactions indicative of the presence of common antigenic substances among the species.

The results with *Ps. syringae* indicate that it may be feasible to use serological tests for identification of isolates of this pathogen. However serological heterogeneity is evident among the cultures, especially with *Ps. marginalis* and *Ps. flourescens*, and this places limitations at present on serological diagnosis of the species of *Pseudomonas* studied.

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AUTOGENOUS NECROSIS OF CABBAGE

Glenn S. Pound and J. C. Walker [1]

In 1946 certain inbred lines of cabbage in a plot inoculated with cabbage mosaic viruses (Cauliflower virus 1 and Turnip virus 1) developed severe necrosis in midseason or later. This was thought to be part of the virus syndrome until the following year when plants of the same lines were found to develop necrosis in the absence of virus infection. Efforts to isolate fungi or bacteria as incitants have failed. Similarly, it has been impossible to demonstrate the presence of a virus by mechanical, aphid, and graft transmission studies. The facts that necrosis increases with inbreeding, that it can be produced in sand culture under insect-proof conditions, and that it is readily covered up by outcrossing to other plants, indicate that it is of genetical origin and it is described herein as an autogenous necrosis.

SYMPTOMS.—Susceptible plants begin to show small necrotic flecks which usually appear first at the distal portion of the leaf blade 6 to 8 weeks after transplanting. Isolated circular necrotic spots and ringspots sometimes appear at other points on the leaf. Severe streaking of the veins also may appear. Progress of the disease continues throughout the rest of the season and symptoms may develop in one of 3 ways: 1) lesions may be so numerous and small as to give leaves a scorched appearance (Fig. 1, B); 2) leaves may be covered with distinct circular necrotic spots up to 1 cm. in diameter, with or without vein streaking (Fig. 1, A); 3) severe extensive necrosis may exist at the base of the fleshy petiole with leaf spotting only in a limited contiguous area (Fig. 1, C).

Affected heads placed in storage often develop an almost complete breakdown of storage tissue and are difficult to bring to flowering. This severe breakdown also occurs late in the season in the field (Fig. 2) in some lines, predisposing plants to bacterial soft rot. When affected heads are brought to flowering severe necrosis and abscission of cauline leaves occur.

EFFORTS TO DETERMINE THE INCITANT.—Numerous attempts were made to isolate pathogenic bacteria or fungi from affected tissue. Every effort was unsuccessful. Numerous attempts to transfer a virus by means of aphids and mechanical inoculations from diseased to healthy cabbage and other plant species were negative. Limited attempts to transmit a virus from cabbage to cabbage by grafting were unsuccessful.

Seed from lots known to show the disease in the field were surface sterilized, soaked in hot water for 30 minutes at 50° C., and sown in quartz sand. The seedlings were transplanted into crocks of quartz sand along with plants from seed lots known to show no necrosis. Since necrosis occurs in the field in the cooler part of the season, pots were kept for 120 days at 16°, 20°, 24°, and 28° C. Insect control was obtained by frequent fumigation. After about 75 days at 16° and 20° the plants of the necrotic lines developed necrosis identical with that in the field. No necrosis developed on control plants at any temperature, or on plants of the necrotic line at 24° and 28°. It appears that expression of the symptoms is a function of either air temperature, or physiological maturity, or both.

INHERITANCE OF NECROSIS.—In taking records individual plants were rated in 1 of 5 severity classes for either leaf spotting, leaf scorching, or petiole necrosis. The disease classes were designated by number from 0 (no signs) to 4 (severe symptoms). A disease index for each plant lot was calculated by a method previously described.[2] When plants were selected for either disease type in successive generations there was a progressive increase in severity until a high degree of symptom expression was uniform throughout the progeny.

Plants showing each type of symptom were crossed with plants from inbred lines which showed no necrosis. In each case selfed progenies from parent plants were tested along with hybrid progenies. In several crosses involving each symptom type, necrosis was entirely absent in the F_1. In the F_2 progenies the percentage of plants showing necrosis was always very low, the necrotic plants being practically always in class 1 and rarely in class 2. By selective inbreeding of necrotic segregates in the F_2 progeny, uniformly highly necrotic lines, similar to the necrotic parent were secured. In Table 1 are shown the results from a cross between a leaf spotting plant (25-888) and a normal plant (25-864). These are typical of results from several crosses. All F_1 plants were normal. About 6 per cent of the F_2 progeny were necrotic. Four necrotic and 3 normal plants were selfed and F_3 progenies were tested. One progeny resembled the normal parent, 3 resembled the necrotic parent, and 2 were intermediate. When 2 leaf-spot necrotic plants (25-196 and 25-194) which yielded progenies with much higher disease indices than 25-888 were crossed, the F_1 index was similar to the 2 parent progenies.

A petiole necrosis plant (25-187) was crossed with

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[2] Pound, G. S., and J. C. Walker. 1951. Mosaic resistance in cabbage. Phytopathology 41: 1083-1090.

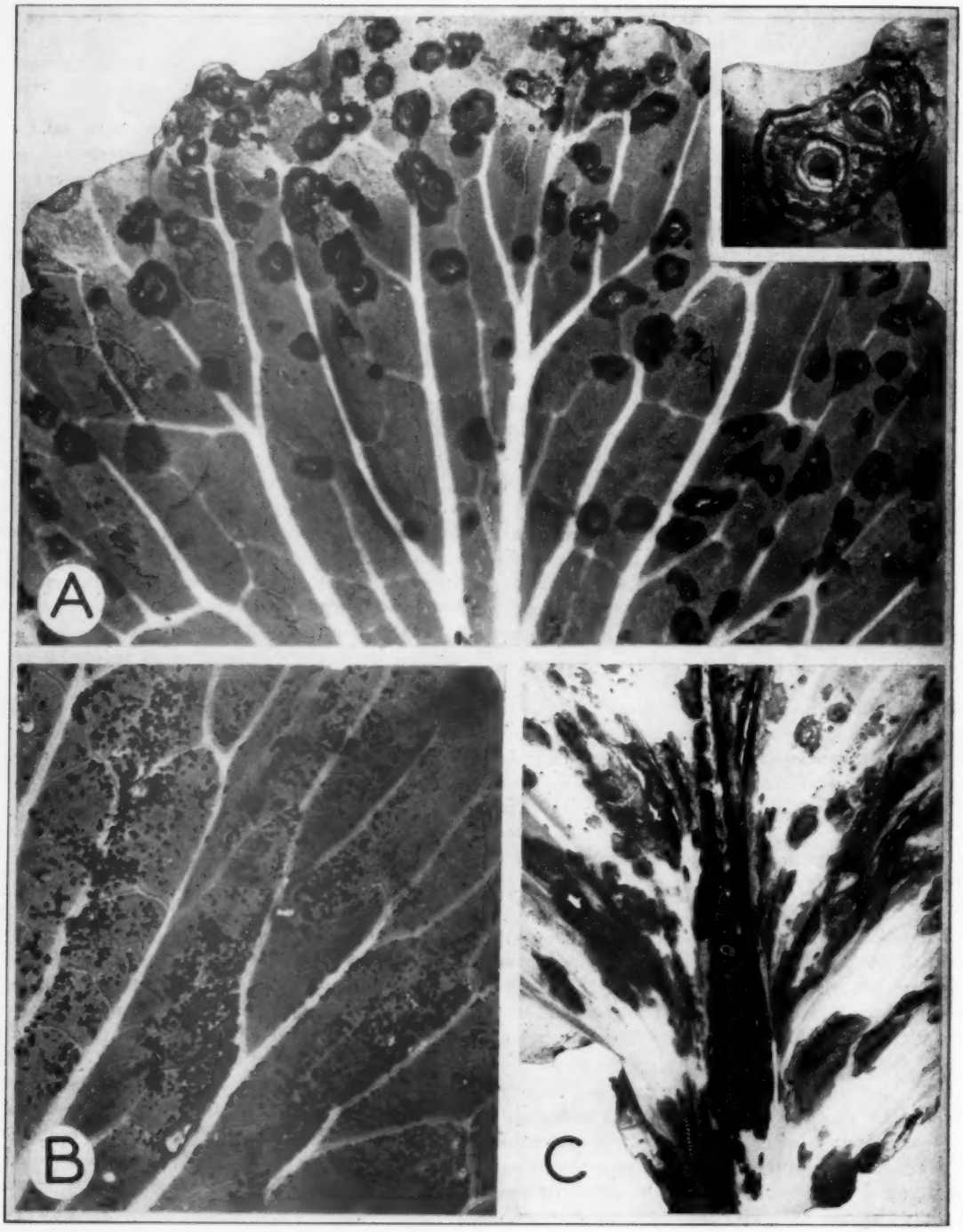

FIG. 1. Types of autogenous necrosis in cabbage. A) general leaf spotting, with enlarged lesion in insert; B) general leaf scorching; C) severe necrosis at base of petiole.

a normal plant (83-635). As with leaf-spot necrosis, petiole necrosis was completely recessive in the F_1 and only about 9 per cent mildly necrotic plants (class 1) appeared in the F_2. This indicated a pattern of inheritance similar to that of leaf-spot necrosis.

The same petiole necrosis parent (25-187) was crossed with a leaf-spot necrosis plant (25-194). Although self progenies from each parent had high petiole-necrosis and leaf-spot necrosis indices, respectively, the F_1 was completely free from petiole necrosis. While all F_1 plants had a very mild type of leaf flecking it was much less conspicuous than with

FIG. 2. Field symptoms on an inbred highly necrotic line of cabbage (right) compared with an inbred line free of necrosis (left).

those of the mildest disease class (class 1) of leaf-spot necrosis progenies. An identical situation occurred when the petiole necrotic plant (25-187) was crossed with a plant showing severe scorching.

DISCUSSION.—Autogenous necrosis in tomato has been shown by Langford[3] to be controlled by a single gene pair, linked with the gene for immunity from *Cladosporium fulvum*. The necrosis types described in this paper have symptoms which overlap those associated with mosaic incited by turnip virus 1 alone or in combination with cauliflower virus 1. Three distinct necrotic types, i.e. leaf spot, leaf scorch, and petiole necrosis, are each recessive to normal and they appear to be controlled by distinct genes. This is shown by the fact that when leaf-spot plants were crossed, the F_1 progeny was similar to selfed progenies of both parents. When leaf-spot or leaf-scorch and petiole necrosis parents were crossed, the F_1 progenies were normal except for a very slight leaf speck. While sufficient crosses and subsequent progenies have not been made to determine the number of genes involved, the data procured indicate that several gene pairs are concerned in the inheritance of each necrotic type.

Inasmuch as the necrotic characters are recessive, the normal cross-pollination followed in cabbage seed production would keep necrotic plants from becoming numerous or very important in commercial populations. However, since a highly necrotic line may be secured by as few as 2 generations of inbreeding of necrotic segregates, it is important to avoid necrotic individuals when inbreeding is being practiced in any cabbage improvement program.

[3] Langford, A. N. 1948. Autogenous necrosis in tomatoes immune from *Cladosporium fulvum* Cooke. Can. Jour. Res. C, 26: 35-64.

SUMMARY

A hitherto unreported disease of cabbage is described. It is an autogenous necrosis found in certain inbred lines of cabbage. It may be manifested by necrotic flecks or spots on leaves followed by breakdown of head tissue or as a severe necrosis of the base of the petioles, resulting in defoliation. The disease appears after midseason under Wisconsin conditions and may become more intense as the season advances. When seedlings of necrotic lines were grown under controlled temperatures in the greenhouse, the disease appeared typically at 16° and 20° C., but did not develop in 120 days at 24° and 28°.

TABLE 1.—*Severity and frequency distribution of plants of various necrotic and non-necrotic lines of cabbage and of progenies of their crosses.*

Progeny		Necrotic reaction of parents	Number plants in severity class 0	1	2	3	4	Disease Index[a]
25-888		Leaf spotting	0	13	13	0	0	37.5
25-864		None	26	0	0	0	0	0.0
25-888 × 25-864	F_1		24	0	0	0	0	0.0
	F_2		78	4	1	0	0	1.8
25-90	F_2		17	18	1	0	0	9.6
25-91[b]	F_2		0	17	7	0	0	32.3
25-92[b]	F_2		0	5	3	0	0	34.4
25-93	F_2		22	0	0	0	0	0.0
25-94[b]	F_2		0	10	8	0	0	37.5
25-95	F_2		19	7	0	0	0	6.7
25-96[b]	F_2		0	8	18	0	0	42.3
25-196[c]		Leaf spotting	0	0	2	17	6	79.0
25-194[c]		Leaf spotting	0	0	7	18	0	68.0
25-196 × 25-194	F_1		0	0	6	12	2	70.0
25-187		Petiole necrosis	0	3	9	13	0	60.0
83-635		None	25	0	0	0	0	0.0
25-187 × 83-635	F_1		25	0	0	0	0	0.0
	F_2		149	15	0	0	0	2.3
25-187		Petiole necrosis	0	3	9	13	0	60.0
25-194		Leaf spotting	0	0	7	18	0	68.0
25-187 × 25-194	F_1	Petiole necrosis	25	0	0	0	0	0.0
		Leaf spotting	0	25[d]	0	0	0	25.0
25-187		Petiole necrosis	0	3	9	13	0	60.0
25-177		Leaf scorching	0	0	3	10	7	80.0
25-187 × 25-177	F_1	Petiole necrosis	24	0	0	0	0	0.0
		Leaf scorching	0	24[d]	0	0	0	25.0

[a] So calculated that 0 = no reaction; 100 = very severe necrosis. [b] These F_3 lines were from selfed F_2 necrotic plants; others were from non-necrotic plants. [c] These were inbred lines of distinctly different origin. [d] Each F_1 plant showed very slight leaf flecking not typical of the parental reaction.

Data presented indicate that expression of necrosis is completely recessive and controlled by a number of genes. The petiole necrosis symptom is apparently controlled by a separate group of genes from those governing leaf spotting.

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FACTORS AFFECTING VIABILITY AND GROWTH OF PHYTOPHTHORA PHASEOLI [1]

R. A. Hyre and R. S. Cox [2]

Downy mildew, caused by *Phytophthora phaseoli* Thaxt., is by far the most destructive disease of the lima bean in Delaware and surrounding states. It is a serious threat to the industry and has been responsible for much shifting of production areas in past years. The yield of an entire area may be greatly reduced in years favorable for disease development, with individual growers frequently suffering losses of 50 per cent or more.

In spite of the seriousness of the disease, growers of the small-seeded lima bean (Henderson Bush types) have not, on the whole, adopted the recommended fungicide programs (10). This reluctance is largely due to: a) the marginal nature of the financial returns, b) cost of the program, c) phytotoxicity, and d) reliance on hot, dry weather to prevent disease development. The need for a more acceptable control program, therefore, has become apparent.

Critical timing of fungicide applications appears to be 1 solution of this problem. Such a program would be greatly facilitated with a reliable disease forecasting and warning service. The value of such services for other diseases, including some downy-mildew types, is well established (19).

The success of any disease forecasting program is dependent upon knowledge of: a) how and where the fungus overwinters, b) means of dissemination, and c) influence of environment upon spore viability, germination, infection, and subsequent sporulation. Such knowledge of the causal organism of downy mildew of lima bean is either inconclusive or lacking in the literature; therefore, a program of research on these aspects of the problem was initiated. Recent information, dealing with the overwintering and dissemination of the fungus, has already been published (6, 9). The purpose of this paper is to present results on that portion of the work concerned with the effect of certain factors on viability and growth of the pathogen.

MATERIALS AND METHODS.—Single zoospore cultures of *Phytophthora phaseoli* were obtained from mass isolates from Thorogreen lima bean collected at Milton, Delaware. Unless otherwise indicated, sporangia used in the various tests were produced on oat agar cultures[3] which were grown at 15°–20° C. for 2 to 3 weeks. They were collected by flooding the cultures with sterile 2 per cent sucrose solution precooled to the particular temperature of the current experiment, and were germinated on cellulose-nitrate-coated glass slides (8) in inverted water-sealed moist chambers. Prior to use the temperature of all glassware was adjusted to that at which the experiment was made. Most tests were repeated 4 to 8 times.

A series of refrigerating incubators were used for most of the controlled temperature studies. All were accurate to ± 0.5° C. except the 1 set for 10° C. which was more variable and tended to be cooler than desired. A water bath, accurate to ± 0.1° C., was used for the longevity studies of sporangia.

EFFECT OF TEMPERATURE ON COLONY GROWTH.—*Phytophthora phaseoli* was first cultured by Clinton in 1905 (3). In later work he obtained best growth on media containing infusion from lima bean seed (4). Leonian (13) maintained stock cultures of the fungus on oatmeal agar. He failed to get appreciable growth on synthetic media except where he added lima bean infusion. This suggested to Saksena and Bhargava (21) that *P. phaseoli* was unable to synthesize certain growth supplements that apparently are present in lima bean infusion. They showed that the fungus required a special amino acid (D-alanine) as a source of nitrogen for growth, and that it suffered from thiamine deficiency. They also found that the ammonium ion was toxic to the fungus.

Based on this information, as well as on results of tests by the authors (11) where 10 different media were used, oat agar was selected for use in the temperature studies. In 4 experiments a total of 7 to 18 90 mm.–Petri dishes, each containing 20 ml. of agar, were used at each temperature. A uniform culture plug, 4 mm. in diameter, was placed in the center of each dish. Test temperatures occurred at 5° intervals and ranged from 5° to 30° C. Average growth for 13 days was determined (Fig. 1A), after an original base period of 6 to 8 days.

Maximum radial growth occurred at 20° C., with nearly as much at 15°; no growth occurred at 5° and 30° C. An exposure period of 2 weeks at 30° C. appeared to be lethal to the fungus, since growth was not observed when plates previously held at 30° were transferred to a lower temperature (15° or 20° C.).

PRODUCTION OF SPORANGIA ON AGAR.—There is gen-

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[2] Pathologist, Division of Mycology and Disease Survey, U. S. Department of Agriculture, Beltsville, Maryland; and Associate Research Professor, Department of Plant Pathology, Delaware Agricultural Experiment Station, respectively.

[3] Oat agar was made as follows: 50 gm. rolled oats was soaked 10 hours or more, blended, then mixed with 17 gm. melted agar. The final volume was 1000 ml. Lima bean agar, equal formula and similarly prepared, also was a satisfactory medium.

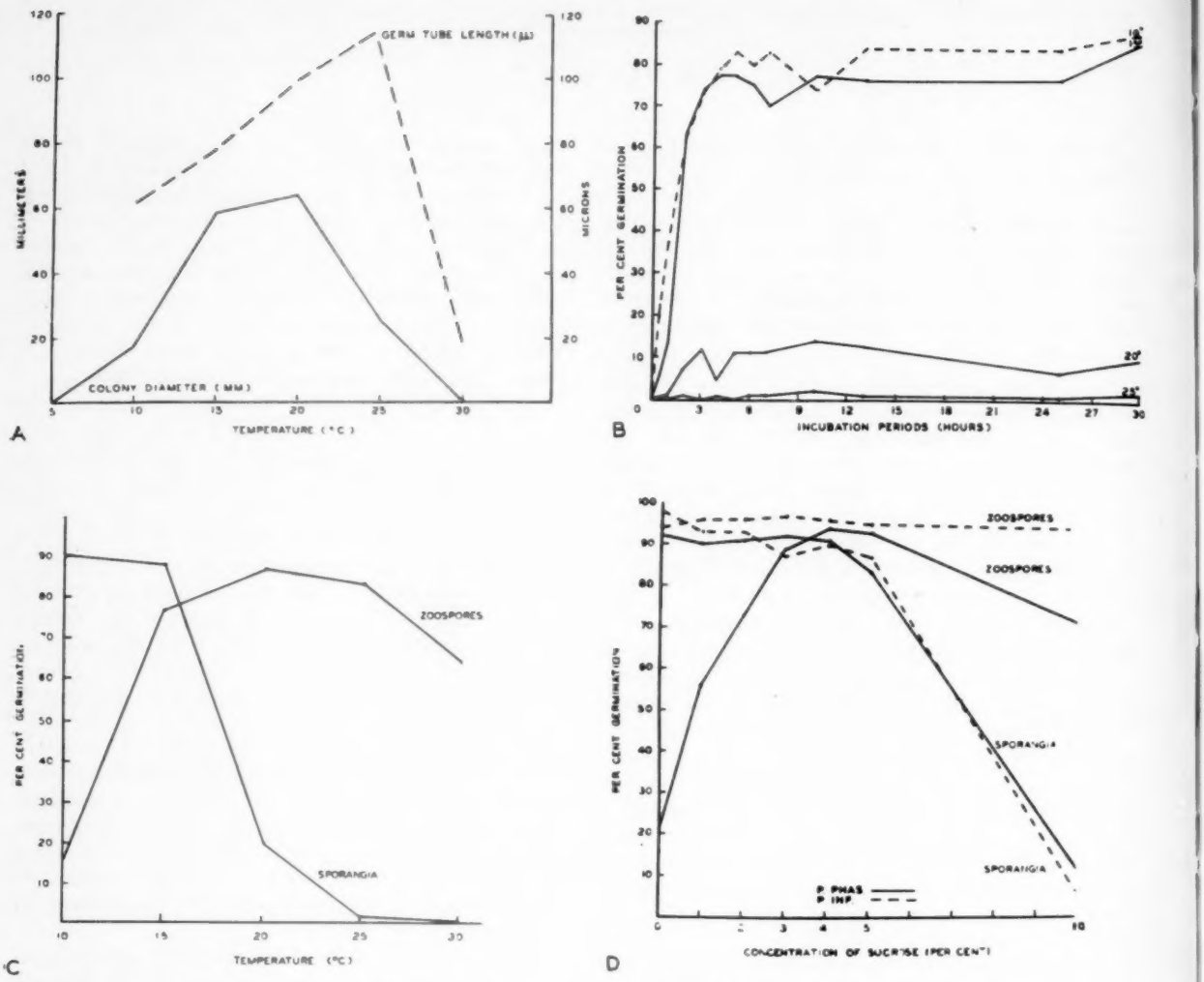

FIG. 1 (expressed as averages). A-C) Effect of temperature on *Phytophthora phaseoli*: A, zoospore germtube length after 24 hours in 2 per cent sucrose solution, and radial colony growth on oat agar after 13 days; B, rate and per cent indirect germination of sporangia; C, zoospore and indirect sporangial germination after 24 hours. D) Effect of rain water and various sucrose concentrations on zoospore and indirect sporangial germination of *P. phaseoli* and *P. infestans*.

eral agreement (4, 20, 24) that, of the culture media tried, most abundant production of sporangia of *Phytophthora phaseoli* occurs on oatmeal agar, followed closely by that on lima bean agar. Similar results were obtained by the authors (11) in limited studies.

Sporangia vary in size depending upon the medium on which they are produced. Leonian (14) found considerable variation in size and shape of sporangia grown on wounded bell pepper fruit compared to those grown on lima bean pods. In the investigations reported here, measurements were made of the length of 450 sporangia, consisting of lots of 50 produced on each of 3 media (oat, lima bean, and Leonian's synthetic) held at each of 3 temperatures (15°, 20°, and 25° C.). The mean lengths were: oat agar, 35.8μ $\pm$ 0.44; lima bean agar, 33.3μ $\pm$ 0.36; Leonian's agar, 31.2μ $\pm$ 0.38.

Sporangia also varied in size when produced at different temperatures. The mean length of 100 sporangia (50 each produced on oat and Leonian's synthetic medium) were: 10° C., 38.6μ $\pm$ 0.51; 15° C., 34.3μ $\pm$ 0.48; 20° C., 32.2μ $\pm$ 0.45; and 25° C., 34.0μ $\pm$ 0.69.

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON VIABILITY OF SPORANGIA.—The length of time sporangia of *Phytophthora phaseoli* remained viable when exposed to different temperatures and relative humidities was determined by employing a modification of the method used by Clayton (2). Glass cover slips were suspended on glass rods attached with paraffin to the glass cover of half-pint fruit jars. Sporangia were transferred with a glass needle to the dry cover slips,

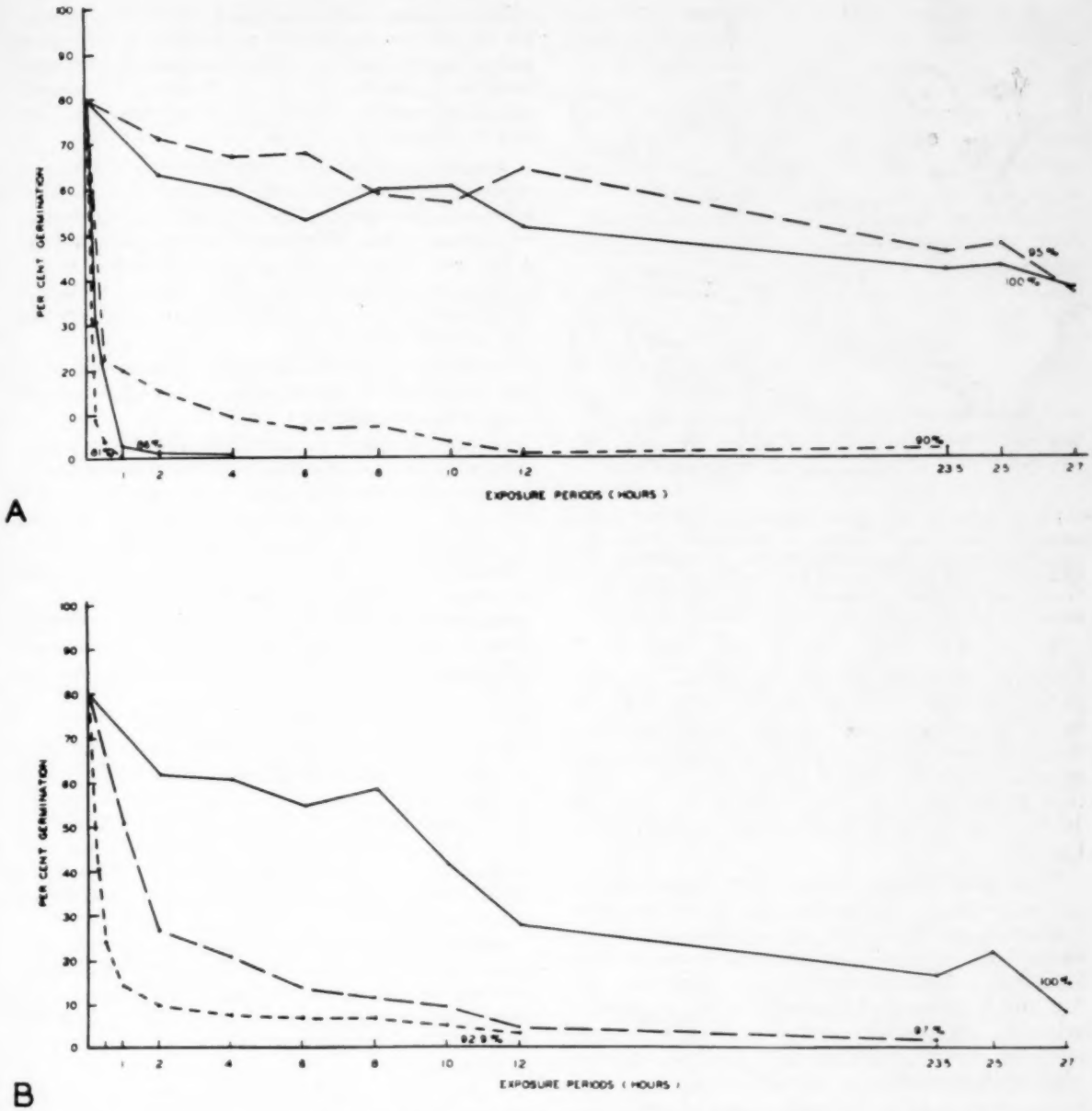

FIG. 2 (expressed as averages). Effect of exposure period at different relative humidities on viability of sporangia of *Phytophthora phaseoli* at: A, 20° C.; and B, 30° C.

which then were suspended over saturated salt solutions in the jars.[4] The jars were then sealed and immersed in a water bath constant to ± 0.1° C. Periodically, 2 cover slips were removed and the sporangia thereon were incubated at 15° C. in 2 per cent sucrose solution plus 0.005 per cent potassium citrate (18) to determine their viability. Data from 4–8 experiments were averaged and plotted in Figure 2.

For convenience of interpretation all experimental data were adjusted to 80 per cent germination for the checks. For example, if a check (unexposed sporangia) had 90 per cent germination and exposed sporangia 45 per cent, the check would be entered as 80 per cent and the exposed sporangia as 40 per cent.

At 20° C. nearly 40 per cent were viable after 27 hours exposure to 95 or 100 per cent relative humidity; 25 per cent were viable even after 50 hours. Longevi-

[4] Other methods of collecting sporangia, such as the use of a cyclone-separator (22) or by touching the sporulating surface of agar discs to dry cover slips, were not successful. Saturated salt solutions used to obtain theoretical relative humidities were: 20° C.—$Na_2HPO_4 \cdot 12H_2O$, 95%; $ZnSO_4 \cdot 7H_2O$, 90%; $KHSO_4$, 86%; $(NH_4)_2SO_4$, 81%; 30° C.—K_2SO_4, 97%; $NH_4H_2PO_4$, 92.9%.

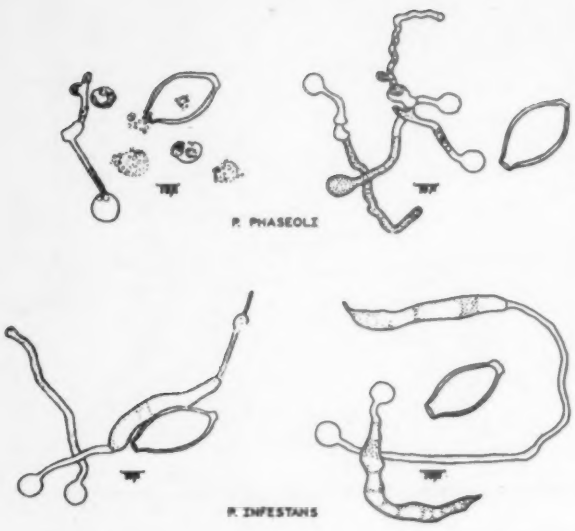

FIG. 3. Effect of rain water (left) and 4 per cent sucrose solution (right) on germination of zoospores of *P. phaseoli* and *P. infestans*. Note disintegration of zoospores of *P. phaseoli* in rain water (upper left); also, appressorial-like bodies and secondary germtubes produced by both organisms.

ty decreased rapidly at lower humidities—less than 10 per cent were viable after 4 hours at 90, 1 hour at 86, or 15 minutes at 81 per cent relative humidity. At 30° C. fewer sporangia were viable after exposures at corresponding humidities and periods of time. Less than 10 per cent were viable after 27 hours at 100, 10 hours at 97, or 2 hours at 93 per cent relative humidity.

These data indicate that distance dissemination of viable sporangia would require high relative humidities —above 90 per cent at 20° and approaching 100 per cent at 30° C. Local spread might occur at lower humidities. However, the chances of infection resulting from locally-spread sporangia under conditions of relatively low humidity probably would be reduced since the chances of the sporangia encountering moist plant surfaces would be lessened.

EFFECT OF SOLUTE CONCENTRATION ON GERMINATION OF SPORANGIA AND ZOOSPORES.—When sporangia of *Phytophthora phaseoli* were germinated in distilled, rain, or tap water many of the resulting zoospores disintegrated (Fig. 3). Melhus (16) reported a similar observation for *P. infestans* (Mont.) deBary and suggested an osmotic phenomenon as the cause. To investigate this further, sporangia of both species were germinated in rain water and in 1, 2, 3, 4, 5, and 10 per cent sucrose solutions. In addition, dilute sodium chloride solutions were tried.

Sporangia.—Normal germination of both species occurred in rain water and in 1, 2, 3, and 4 per cent sucrose solutions, but it was somewhat less in 5 per cent solution, and much less in 10 per cent solution (Fig. 1D).

Dilute sodium chloride solution was toxic to sporangia of both species. Indirect germination in rain water and in ¼, ½, and 1 per cent sodium chloride solutions in 1 experiment was as follows: *P. phaseoli* 68, 12, 2, and 0, respectively; *P. infestans* 82, 1, 0, and 0, respectively.

Zoospores.—A marked disintegration of zoospores of *P. phaseoli* occurred in rain water. In sucrose solutions, disintegration became progressively less as concentration was increased, reaching a minimum in 4 per cent (Fig. 3). Germination increased as disintegration decreased (Fig. 1D). However, the reduced germination in 10 per cent solution was not due to disintegration.

About 95 per cent germination of zoospores of *P. infestans* occurred in rain water and in all concentrations of sucrose solutions.

EFFECT OF MOISTURE AND TEMPERATURE ON GERMINATION OF SPORANGIA.—Thaxter (23) discovered early that sporangia of *Phytophthora phaseoli* may germinate in 2 ways: either indirectly by formation of about 15 zoospores, or directly by means of a germ tube.

Moisture.—Free water is necessary for sporangial germination. Numerous observations were made of sporangia suspended on dry cover slips over distilled water in sealed half-pint jars immersed in a constant temperature bath. In no case was germination observed in such saturated atmospheres unless the sporangia were in free water.

Temperature.—a) *Indirect Germination.* The effect of temperature on indirect germination of sporangia in 4 per cent sucrose solution was determined by employing the standard glass-slide technique (1). Germination counts were made from a different slide at each examination at various intervals up to 30 hours. From these data the rate and the final per cent germination were determined.

The rate of germination was faster at the lower temperatures (Fig. 1B). At 10° and 15° C. germination had started within 30 minutes and was virtually completed within 3 hours. At 20° C. germination had just started in 1 hour, but likewise was completed in about 3 hours.

Perhaps more significant was the effect of temperature on final percentage of germination (after 24 hours). This was determined in 2 series of tests. Four test temperatures, 10°, 15°, 20°, and 25° C., were employed in each series. In addition a fifth, 30° C., was added in the second series. Maximum germination occurred at 10° and 15° C., frequently being as high as 95 per cent. At 20° C. total germination was considerably reduced; the maximum observed was 30 per cent. The average germination of 2 replicates in the first series was 10 per cent (Fig. 1B), and that of 3 replicates in the second series was 20 per cent (Fig. 1C). At 25° C. germination averaged about 2 per cent; none occurred at 30° C.

b) *Direct Germination.* Direct germination of sporangia of *P. phaseoli* seldom exceeded 4 per cent at any temperature and averaged about 1 per cent at 25° C.

In this respect, the fungus appears to differ markedly from *P. infestans* but more direct comparisons are needed. Melhus (16) and Crosier (7) reported an average of 22 to 30 per cent direct germination of sporangia of *P. infestans* at the optimum temperature of 23° to 24° C.

EFFECT OF TEMPERATURE ON GERMINATION OF ZOOSPORES AND ON GERMTUBE ELONGATION.—After a period of swarming, zoospores come to rest, round up, and become invested with a cell wall. Germination then occurs by means of a germtube.

For the germination experiments, zoospores were obtained by incubating sporangia in a precooled sucrose solution at 10° C. for 2 to 3 hours. In this time 80 to 95 per cent of the sporangia had germinated. The resulting zoospore suspension was then used for germination studies, employing the standard glass slide technique (1). In the first tests 4 per cent sterile sucrose solutions were used. Objectionable bacterial growth, especially at the higher temperatures, hindered germination of the zoospores. This problem was largely eliminated in subsequent tests by employing 2 per cent non-sterile sucrose solution (25).

Motility.—The duration of motility of zoospores decreased as temperature increased. Some zoospores were motile for at least 24 hours at 10° C., 12 hours at 15°, 6 hours at 20°, and less than 3 hours at 25° and 30° C.

Germination.—The rate and final percentage of germination were determined at 5 temperatures. The rate increased with a rise in temperature up to 25° C., then decreased at 30° C. This is illustrated by 1 test where the percentage of germination was 1, 3, 30, 41, and 21 after 6 hours exposure to 10°, 15°, 20°, 25°, and 30° C., respectively.

The final average percentage of germination (after 24 hours) for 3 experiments is given in Figure 1C. The optimum temperature appeared to be about 20° C., although the per cent germination was nearly as high at 15° and 25° C. Less germination occurred at 10° and 30° C. The optimum temperature (about 20° C.) for germination of zoospores is higher than that for indirect germination of sporangia (10°–15° C.).

Germtube Elongation.—Average length of 50 germtubes after 24 hours at each of 5 temperatures is recorded in Figure 1A. Growth rate increased with a rise in temperature up to 25° C. Germtubes appeared but grew very slowly at 30° C.

The growth curve for germtubes is similar to that for radial colony increment except that in the latter case no growth occurred at 30°, and maximum growth occurred at 20° instead of 25° C.

FORMATION OF OOSPORES.—Clinton (3) was the first to find oospores of *P. phaseoli*. They were produced both in plant material and in culture. Later he investigated the validity of his theory of "distinct mycelial strains," postulating the necessity of both strains being present for the production of oospores (4). His results tended to disprove this theory, however, since he always had oospores produced in cultures from "isolated spores" or "single isolated threads" from the margin of colonies.

In the present studies the authors worked with several single zoospore cultures isolated by the Keitt method as modified by Cox (5). Oospores were readily produced by these isolates, demonstrating homothallism for this fungus.

The number of oospores produced was influenced both by temperature and medium employed. They were produced most abundantly at the higher temperature although they were present at all temperatures studied (10° through 20° C.). Lima bean agar yielded about 4 times as many oospores as did oat agar as determined by examination of several comparable cultures.

The size of oospores and oogonia was determined when they were produced on 16-day-old cultures at 20° C. on lima bean agar. The mean diameter of 100 oospores was 19.2μ, the range being 14.4 to 26.6μ. The corresponding figures for oogonia were 23.7μ, and 16.2 to 35.6μ.

DISCUSSION.—In spite of their close relationship (13), there are distinct biological differences between *P. phaseoli* and *P. infestans* which might influence the epiphytology of the diseases caused by them.

The most obvious and perhaps most significant difference is that of the ready production of oospores by *P. phaseoli* both in nature and in culture, as compared to the rare known occurrence of oospores of *P. infestans* (24). This fact, apparently, influences persistence of the organisms in certain areas. Initiation of late blight of potato and tomato appears to be dependent upon introduced primary inoculum from cull piles, transplants, etc.; whereas, *P. phaseoli* is capable of overwintering in the soil (6).

According to Crosier (7), sporangia of *P. infestans* remained viable somewhat longer at relatively low humidities than was found to be the case with *P. phaseoli* in the present study. However, Crosier used sporangia attached to diseased plants during the exposure period, while the authors obtained sporangia of *P. phaseoli* from agar cultures.

The 2 organisms appear to differ in the motility period and the optimum germination temperature of their zoospores. In the present work with *P. phaseoli*, motility was still apparent after 24 hours at 10° C.; while little was observed after 12 hours with *P. infestans* (7, 16). Also, it appears that optimum temperature for zoospore germination is higher for *P. phaseoli* (about 20° C.) than for *P. infestans* (11° to 15° C.). However, this difference might be due to the germinating media used—sucrose solution for *P. phaseoli* and water for *P. infestans*.

Another physiological difference between zoospores of the 2 organisms is indicated by disintegration studies. A much higher percentage of the zoospores of *P. phaseoli* disintegrated in water than was the case with *P. infestans*. This might be due to their varying inherent capacity to take up water and still

retain their cellular identity. Such could result from differences in solute concentration, permeability, and/or elasticity and tensile strength of the cellular membrane. The significance of this phenomenon under field conditions is speculative since it is likely that moisture which occurs on plant tissues contain plant secretions (17) and other impurities.

Considerably less direct sporangial germination in the case of *P. phaseoli* appears to be still another important difference between the 2 organisms, indicating that the development of downy mildew of lima bean may be more adversely affected than that of late blight of potato and tomato under conditions where infection may result from direct germination.

It would appear that downy mildew of lima bean is a disease excellently adapted to the development and maintenance of a forecasting service. Disease severity can be expected to vary considerably because of the sensitivity of the causal organism to its environment. When it is absent, or nearly so, fungicides are not applied for any other disease; thus, their use is predicated solely on the anticipated occurrence of downy mildew. Forecasting should be facilitated by the relatively uniform geographical area in Delaware and surrounding states devoted to the production of lima beans.

SUMMARY

Maximum radial growth of *Phytophthora phaseoli*, on oat agar, occurred at 20° C. Growth at 15° was nearly as good. None occurred at 5° and 30° C. Two weeks exposure to 30° C. apparently was lethal to the fungus.

Number and size of sporangia varied depending upon the medium and the temperature at which they were produced.

At 20° C. nearly 40 per cent of sporangia were viable after 27 hours exposure to 95 or 100 per cent relative humidity, and 25 per cent were viable after 50 hours. Less than 10 per cent were viable after 4 hours at 90, 1 hour at 86, or 15 minutes at 81 per cent relative humidity. At 30° C. less than 10 per cent were viable after 27 hours at 100, 10 hours at 97, or 2 hours at 93 per cent relative humidity.

Ten per cent sucrose solution reduced germination of sporangia. Lesser concentrations had no effect. Dilute sodium chloride solution was toxic to sporangia of both *P. phaseoli* and *P. infestans*.

Due to disintegration, germination of zoospores of *P. phaseoli* in rain water was less than 30 per cent. It increased to over 90 per cent in 4 and 5 per cent sucrose solutions, then decreased to about 70 per cent in 10 per cent solution. Little disintegration of zoospores of *P. infestans* was observed.

Free water is necessary for germination of sporangia of *P. phaseoli*.

Direct germination of sporangia seldom exceeded 4 per cent and averaged about 1 per cent at 25° C.

At optimum temperatures indirect germination of sporangia had started within 30 minutes and was virtually completed in 3 hours. Average percentage of germination at 10°, 15°, 20°, 25°, and 30° C. was about 84, 80, 13, 1, and 0, respectively.

Some zoospores were motile for at least 24 hours at 10° C., 12 hours at 15°, 6 hours at 20°, and less than 3 hours at 25° and 30° C.

Rate of zoospore germination and germtube elongation increased with temperature to 25° then decreased at 30° C. After 24 hours at 10°, 15°, 20°, 25°, and 30° C., percentage of germination was 16, 77, 87, 83, and 64; average germtube length in microns was 62, 79, 99, 114, and 19, respectively.

Single zoospore isolates produced oospores in culture, demonstrating homothallism for *P. phaseoli*. The number increased with temperature; more occurred on lima bean agar than on oat agar.

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RELATION OF WOUND PERIDERM IN GLADIOLUS CORMS TO PENETRATION BY FUSARIUM OXYSPORUM F. GLADIOLI [1]

Barton H. Marshall, Jr.[2]

One of the earliest reports of a gladiolus disease appeared in 1909 (7). The symptoms described indicate that this was the disease now known as yellows, caused by *Fusarium oxysporum* f. *gladioli* Snyder & Hansen. Today it is the most destructive disease of gladiolus in the warmer sections of this country.

Inasmuch as the greatest corm loss from Fusarium occurs during storage, better control during this period is needed. One of the requirements for successful corm storage is the quick repair of injuries received during harvest so that no exposed tissues remain as infection courts for micro-organisms. Given the proper environment, wound periderm or cork barriers develop over injured exposed tissues and sometimes around dead tissue killed by attack of micro-organisms.

From the beginning, control recommendations for yellows and other corm rots have dealt largely with eradicant fungicides either as dusts or dips. However, results have been erratic and disadvantages such as corm injury, delayed blooming, and reduced flower yields may be involved. The writer has observed that wettable Spergon dust (tetrachloro-para-benzoquinone) applied to the cut surface of gladiolus corms causes destruction of numerous layers of cells under the cut surface and delays the rate and extent of the wound-healing processes (Fig. 1c).

A treatment prior to storage involving no chemicals is being used by some growers with good results. It consists of a heat treatment or curing process at digging time both before and after the corms are cleaned. Freshly dug corms are placed on screen or slat-bottomed trays in an aerated room at a temperature of 80–95°F. for 3 or 4 days. They are then removed, allowed to dry further in common storage for several weeks, cleaned and returned to the heated room for another heat treatment before being stored for the winter. Gould (4) states that proper curing immediately after digging may be more effective in controlling rots than fungicidal treatments at that time.

Normal healing of a cut surface, however, involves formation of wound periderm which requires a certain amount of moisture not supplied in quick drying treatments. Growers in the State of Washington using this curing treatment prior to storage believe a relative humidity of above 50 per cent to be adequate, while Southern California growers prefer to "hold the humidity down to 60 per cent" (3). Priestly and Woffenden (9) state that the hard dry layer of tissues which forms in dry air cracks very easily and is of no protective value. According to Weimer and Harter (10), when cut surfaces dry too rapidly, surface cells die, forming a hard surface layer beneath which periderm seldom develops. On the other hand, when the escape of moisture from the wound surface is retarded, a cork layer develops normally beneath the injured surface.

Artschwager (1) showed that wound cork in potato develops rapidly when the humidity is held practically at saturation, but that development of this protective layer is considerably slowed or even inhibited at lower humidities. Lauritzen and Harter (5) were unable to obtain infection of sweet potato at temperatures favorable for infection if the relative humidity was 90 to 100 per cent, but infection developed rapidly if the humidity dropped to 80 per cent. This has been attributed to the formation of a wound periderm at the higher humidities.

As shown by Artschwager and Starrett (2), gladio-

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lus corms respond to wounding by suberization of the cut surface and subsequent development of wound periderm. Since both processes are greatly favored by high temperature and high relative humidity, it seemed reasonable to assume that better protection might be afforded gladiolus corms during storage if the pre-storage heat treatment now recommended were supplemented with a high relative humidity. Experiments were designed to test this assumption and, at the same time, to compare the effectiveness of 2 of the best pre-storage fungicidal treatments.

MATERIALS AND METHODS.—Corms of 2 varieties of gladiolus, Picardy and Corona, chosen for their extreme susceptibility to *Fusarium*, were used in these experiments. The *Fusarium* used for all inoculations was isolated by the writer on March 30, 1951, from lesions in Florida-grown Picardy corms typical of those caused by *F. oxysporum* f. *gladioli*. Earlier tests had shown this isolate to be highly pathogenic.

Inoculum was prepared by growing the organism on Petri plates of potato-dextrose agar. When the plates had been completely covered with mycelium, the spores were "harvested" by adding sterile distilled water and agitating the surface of the mycelium to remove as many spores as possible. This spore suspension was used for inoculation. A suspension was obtained from a fresh growth of the organism for each day's inoculation.

A sliding microtome was used to cut sections of corms for microscopic examination. Sections were stained with a variation of the ammoniacal gentian violet stain for suberin used by Artschwager (1). Following staining and examination corm sections were preserved in excellent condition for as long as 2 months, for comparative purposes, by simply placing them in distilled water in covered Petri dishes and storing in a refrigerator.

The moist chamber used in the pre-storage treatments was a large metal-lined chamber having a capacity of 4 cu. ft. To maintain a constant relative humidity, water was kept flowing over the floor of the chamber. A drain returned it to a large tank, from which it was recirculated by an electric pump. Humidity in both this chamber and the small glass moist chambers used in the laboratory was found to be 95 per cent as measured with a Serdex hygrometer. Temperature of the water in the tank was thermostatically controlled and the chamber was maintained at a fairly constant temperature of 95°F., never going higher and seldom dropping more than 3° in a 24 hour period. Temperature in the chamber was recorded automatically with a Bristol recording thermometer.

EXPERIMENTAL RESULTS.—*Effect on the corm of conditions favoring cork formation.*—Numerous workers have established the fact that wound periderm is an effective barrier to micro-organisms. It was thus necessary to determine the number of days' exposure to a high temperature and high relative humidity required for a cut corm surface to form a periderm capable of preventing *F. oxysporum* f. *gladioli* from effecting penetration. A temperature of 85°F. was chosen, as rapid periderm formation has been reported at this temperature in potato and as it also is the optimum temperature (6) for growth of the pathogen.

On September 25, 1951, 27 medium-size, healthy Florida-grown corms of the variety Picardy were selected, surface sterilized by dipping in a 1:1000 $HgCl_2$ solution for 5 minutes, washed with sterile water, air dried and cut into quarters with a sharp sterile blade. Nine moist chambers were prepared by placing several thicknesses of moist paper toweling in the bottom of each chamber and autoclaving the chambers. Twelve corm quarters, taken at random, were then placed in each of the 9 moist chambers. Inoculation was accomplished by placing several drops of a spore suspension from a sterile pipette on the cut surface of each corm so that all of the cut surface was moistened with inoculum. One chamber of corm quarters was inoculated immediately and 1 chamber each day thereafter for 7 consecutive days. One chamber was left noninoculated as a control. All chambers were immediately stored in an incubator at a constant temperature of 85°F.

Each day after inoculation for 7 consecutive days 1 corm quarter from each inoculated chamber, together with 1 quarter from the noninoculated chamber, was removed. Sections 15 μ in thickness were then made perpendicularly through the injured surface and all sections stained with ammoniacal gentian violet. After being stained the sections were examined for evidence of suberin, wound periderm, and penetration by the pathogen. Results are in Table 1 and Figure 1A. Corms inoculated immediately offered no noticeable resistance to the pathogen and were rapidly invaded, at least 1/3 of each corm quarter being rotted in 1 week. Those inoculated 1, 2, or 3 days after being cut showed an initial resistance in the form of 2–3 layers of suberized cells and 1–5 layers of periderm, but these layers formed only in isolated spots under the cut surface and were either eventually penetrated or bypassed by the advancing rot. In the 4-day test, suberin and periderm layers were of sufficient strength to prevent penetration of *Fusarium* in all but 2 or 3 spots, while corms inoculated 5, 6, and 7 days after being cut had formed protective layers capable of completely preventing penetration.

Sections from each chamber were also stained and examined 14 days after inoculation. Sections initially infected were further rotted in about the same progression while those stored 5, 6, and 7 days before being inoculated were still unpenetrated. Contaminating organisms made further examination impracticable.

Choosing the test temperature.—Though 85°F. proved an excellent temperature for formation of wound periderm, it was also the optimum temperature for the growth of *Fusarium*. A temperature optimum for corking and inhibitive to growth of this particular organism was desired. From Massey's work (6) it was learned *Fusarium* made little growth at 95°

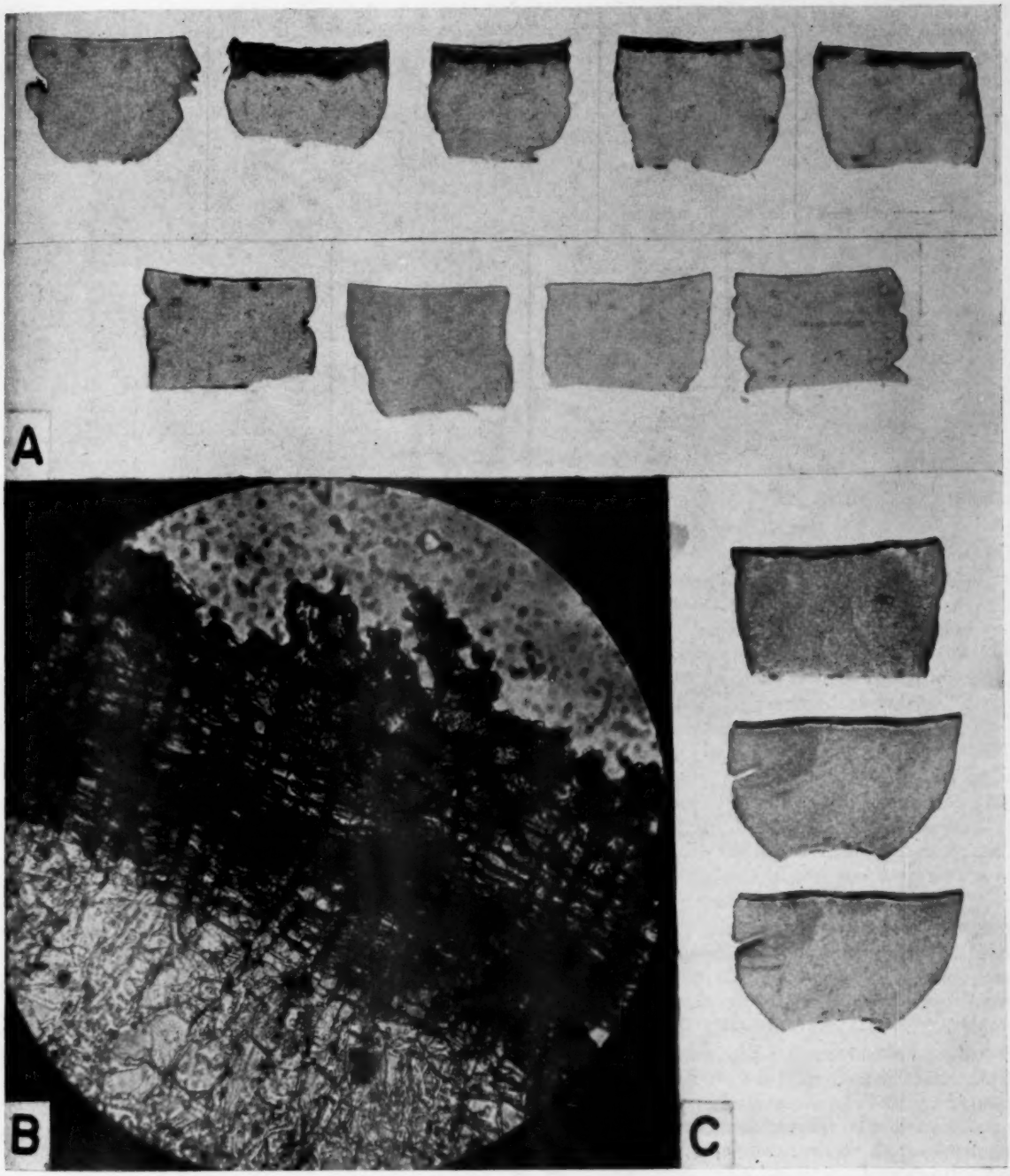

FIG. 1. Wound periderm formation in gladiolus corms var. Picardy. A) Corm sections showing the effectiveness of wound cork in preventing penetration by *Fusarium oxysporum* f. *glodioli*. Top, Left to Right respectively: check, inoculated immediately and after 1, 2, and 3 days' incubation. Bottom, Left to Right respectively: inoculated after 4, 5, 6, and 7 days' incubation. The thin line of stained outermost cells in the unrotted sections are suberized; a well-developed periderm lies just beneath. B) Cross-section of a Fusarium rot lesion (natural infection) showing "walling off" of the pathogen by a well-developed wound periderm. C) Corm sections showing Spergon injury. The first 10 to 20 cells layers below the cut surface have been killed. The dark line forming below these dead cells is a suberin layer, beneath which some periderm is forming.

and none at 100° or 105°F. Hence, to determine the best temperature to use, an experiment was set up as before except that the chambers were incubated at 90°, 95°, 100°, and 105°F. rather than at 85°F. alone. Quartered corms were placed in each of 8 moist chambers and 2 chambers placed at each of the 4

TABLE 1.—*Period needed at 85°F., and 95 per cent relative humidity for cut surface of Picardy corms to form a cork barrier capable of preventing penetration by Fusarium oxysporum f. gladioli*

Days before inoculation	Incubation after inoculation (days)	Number of cell layers suberized	Number of layers of periderm	Approximate number of cell layers penetrated by Fusarium
0	1	0	0	2
	3	0	0	6
	5	0	0	20
	7	0	0	65
1	1	2	0	0
	3	0	0	8
	5	0	0	25
	7	0	0	40
2	1	2	0	0
	3	3	1	10
	5	3	2	20
	7	3	2	30
3	1	3	3	0
	3	3	5	10
	5	3	5	15
	7	3	5	30
4	1	3	3	0
	3	3	6	0
	5	3	7	10
	7	3	7	15
5	1	3	4	0
	3	3	7	0
	5	3	7	0
	7	3	8	0
6	1	4	6	0
	3	4	6	0
	5	4	8	0
	7	4	8	0
7	1	5	6	0
	3	5	8	0
	5	5	8	0
	7	5	8	0
Noninoculated	1	1	0	0
	3	1	1	0
	5	3	3	0
	7	3	6	0

temperatures; at each temperature there was 1 inoculated and 1 noninoculated chamber. Inoculations were made as before except that all corms were inoculated immedately after being cut. Results are in Table 2.

After 1 day suberization was good in the noninoculated corms at both 90° and 95°F. although slightly spotty at 95°F. These temperatures were about equally favorable for periderm formation; the first signs appearing on the third day after the corms were cut. Inoculated corms at 90°F. were, like those at 85°F., quickly rotted. At 95°F. conditions were apparently unfavorable for the organism and corms were penetrated more slowly.

At 100°F. suberization was spotty and poorly developed. Periderm did not form until after 7 days and this, too, was weak and of little protection to the corm. After 11 days at 105°F. there was some callus in the first 2 cell layers. Neither suberin nor periderm could be detected. In inoculated corms, the organism continued to make an irregular, limited penetration of as many as 6 cell layers at 100°F. but was completely stopped at 105°F.

Since both suberization and periderm formation appeared to take place about equally well at 95° and 85°F. and the former temperature was found to impede the growth of *Fusarium*, a temperature of 95°F. was used in further tests.

Pre-storage tests.—Corms were dug at Beltsville, Maryland, October 15, 1951. Weather conditions were excellent for digging and the corms were removed from the ground with a minimum of adhering soil and moisture. Allowing a large margin for unusable corms, 1500 of the variety Picardy, ½ extra large (about 3-in. diameter) and ½ medium size (1½–2-inch diameter) were selected and stored in slatted trays on a greenhouse bench. Temperature in the storage greenhouse was subject to a variation from an occasional high of 80°F. on sunny days to a low of 60°F. at night. On harvest day, 112 large and 112 medium size corms were divided into 7 lots of 32 corms each, 16 large and 16 medium. Five lots

TABLE 2.—*Suberization, wound periderm formation, and Fusarium rot of Picardy corms at various temperatures*

Incubation temperature °F.	Period of incubation (days)	Uninoculated corms Cell Layers (Approx. no.) Suberin	Periderm	Rot	Inoculated Corms Cell layers (Approx. no.) Suberin	Periderm	Rot
90	1	½	0	0	0	0	1
	3	1	0	0	0	0	5
	5	2	3	0	0	0	20
	7	2-3	5	0	0	0	50
95	1	0-½	0	0	0-½	0	0
	3	1	1	0	0	0	5
	5	2	4	0	0	0	12
	7	4	6	0	0	0	15
100	1	0-½	0	0	0-½	0	0
	3	½-1	0	0	0	0	3
	5	1-2	0	0	0	0	5
	7	1-2	2	0	0	0	6
105	1	0	0	0	0	0	0
	3	0	0	0	0	0	0
	5	0	0	0	0	0	0
	7	0	0	0	0	0	0

were cleaned immediately and 2 lots were left uncleaned. Each lot was then given 1 of 7 treatments as follows:

Treatment 1. Corms were completely cleaned by first removing the adhering cataphyllary leaves and parent corm and then dipping them in a suspension of Fusarium spores. They were then dried as rapidly as possible with an electric fan. Half of both size corms were bruised by shaking them several times in a wooden box following which the base of each corm was pressed in wettable Spergon dust (48 per cent tetrachloro-para-benzoquinone). The corms were then placed in screen bottom trays (24″ × 18″), which were divided into quarters so that each variation of the treatment might be kept separate.

Treatment 2. Same as 1 expect that corms were soaked for 1 hour in a solution of Dow 9-B (32 per cent zinc trichlorophenate) at the rate of 3 pounds in 100 gal. of water rather than treated with Spergon.

Treatment 3. Uncleaned corms were placed in a forced draft oven for 36 hours at 85°F. They were then stored at room temperature (70°–85°F.) for 14 days (until dry enough to be easily cleaned). Following cleaning, corms were inoculated and air dried, ½ of both sizes bruised, and all corms returned to the oven for 24 hours before final storage.

Treatment 4A. Same as 1 and 2, but no chemicals were used. Corms were placed in the moist chamber at 95°F. and 95 per cent relative humidity. At the end of 7 days, they were removed and stored.

Treatment 4B. Same as 4A, but inoculation was made *after* removal from the moist chamber.

Treatment 4C. Uncleaned corms were placed in the moist chamber for 7 days and then removed to a greenhouse bench for drying. After drying for 14 days corms were cleaned, inoculated, and dried, ½ of each size bruised, and stored.

Treatment 5. Check lot: corms were cleaned, inoculated, and dried, ½ of each size were bruised, and all were stored.

Similar lots were treated 5, 10, 20, and 30 days after digging. A total of 1120 corms was used.

There was some loss from *Fusarium* in the field from which these corms were dug, but it seems likely that only a small percentage of corms carried a natural infestation. To insure that the corms being used were contaminated with *Fusarium* and that the treatments would receive an adequate test, all corms were dipped in a spore suspension prepared as before. Each lot of treated corms was stored in separate screen bottomed trays on a side bench near the ventilators in a cool greenhouse, (60°–70°F.). The 5 lots in each of the 7 treatments, representing treatment on digging day and 5, 10, 20, and 30 days after digging, were stacked one above the other. One end of the bottom tray rested on the side of the bench to permit some circulation of air and each stack was loosely covered with a piece of black asphalt paper to exclude most of the light. This was done to promote further development of periderm during storage (8). The experiment was terminated 2 months after inoculation of corms treated 30 days after digging.

The first striking effect was the extreme injury to corms soaked in Dow 9-B on digging day. Damage caused by this fungicide covered most of the surface of the corms, changing their normal yellow-orange color from light to dark brown. Corms cleaned on digging day were the most severely injured, the injury decreasing progressively as the corms received longer periods of curing before cleaning. From observation only, heat supplemented with a high humidity appeared to be somewhat superior to the heat of a forced draft oven alone (Treatments 4C and 3 respectively). These treatments cannot be accurately compared, however, since the temperature in the moist chamber was maintained at 95°F. while the highest temperature that could be obtained from the oven was 85°F. Corms treated with Dow 9-B on digging day

TABLE 3.—*Mean number of mummied Picardy corms in pre-storage tests*

Interval (days) between digging and treatment	Mummied corms in individual treatments[a]							Interval means[c]
	1	2	3	4A	4B	4C	5	
0	16	16	0	16	8	0	16	10.3
5	16	16	0	16	0	0	16	9.1
10	11	4	0	16	1	0	11	6.1
20	12	7	0	16	1	0	10	6.6
30	10	5	0	16	1	0	8	5.7
Treatment means[b]	12.9	9.5	0.0	16.0	2.0	0.0	11.9	

[a] Data based on 160 corms per treatment. A total of 1120 corms was used. These figures represent the mean number of both large and small and bruised and unbruised corms. Thus, with a total of 32 corms in each of these categories, the number 16 represents 100 per cent mummification.

[b] L.S.D. at 5 per cent level = 3.4

[c] L.S.D. at 5 per cent level = 1.3

and 5 days after digging, as well as those treated with Spergon, quickly rotted and were reduced to mummies within 4–6 weeks. Untreated corms and those receiving other treatments on those dates, with the exception of those being placed in the moist chamber after inoculation (Treatment 4A), rotted a little more slowly. In all treatments, considerable rotting occurred but was retarded to some degree on the later treating dates. Table 3 shows the mean number of mummied corms as affected by treatment and treating date. As would be expected, bruised corms generally rotted more quickly than did those corms not bruised, but for statistical purposes the differences between the 2 groups was slight enough for them to be considered 1 group.

"Walling off" infection.—To investigate the ability of corms to "wall off" a Fusarium infection, an experiment was set up with diseased rather than healthy corms. Forty-eight Picardy corms both small and large were selected. No more than ¼ of any corm was rotted when the experiment was begun, the rotted area being much less in most cases. The corms were then divided into 2 groups of 24 corms each equal as to size of corms and extent of rot, and surface sterilized for 5 minutes in a 1:1000 HgI_2 solution to destroy any secondary organisms that might be favored by incubation. Both groups were then placed in separate screen-bottomed trays, 1 of which was incubated for 7 days at 95°F. and 95 per cent relative humidity before storage and the other stored immediately. The same greenhouse bench was used for storage. Extra corms were provided in each tray so that samples could be taken periodically and inspected for periderm. Several sections were cut from both small and large corms through both small and large lesions at 2-day intervals for 14 days following storage. Sections were then stained and examined for periderm along the advancing margins of the rot. In corms which received no heat treatment there was no sign of periderm forming to "wall off" the invading organism. In those exposed for 7 days to a 95°F. temperature and 95 per cent relative humidity there was definitely some clearing of starch in advance of the rot, which is preliminary to periderm formation and, in a few sections at least, there was possibly some evidence of periderm. There was not a well defined layer of periderm capable of "walling off" the advancing rot in any corms examined.

Although no solid cork barrier was formed, there was evidently sufficient inhibition by those corms corked to slow advance of the rot. With few exceptions, corms receiving no treatment prior to storage were reduced to mummies in 2 months, while those maintained 7 days at 95°F. and 95 per cent relative humidity rotted more slowly.

The same experiment was performed with the variety Corona. Examination again failed to show an inhibitive periderm forming along the advancing margins of the rotting tissue. Differences in the progress of rot between those corked and those uncorked, were less marked than in the variety Picardy. This might be attributed to the fact that Picardy corms were cleaned several weeks earlier than Corona. Thus the Corona corms had additional time to cure before cleaning.

DISCUSSION.—In pre-storage tests, it was particularly surprising that inoculated corms soaked for 1 hour in Dow 9-B rotted so rapidly. This seems to indicate that latent infections of natural origin probably occurred in all corms, and those corms treated with Dow 9-B shortly after being harvested (as well as those treated with Spergon) were injured, weakened, and thus rendered more susceptible.

Even though growth of the gladiolus *Fusarium* is almost negligible on potato-dextrose agar at 95° F., inoculated corms incubated at this temperature showed considerable infection. It appears that the maximum temperature for growth of the organism is higher when the organism is associated with the suscept than when it is grown on artificial media. Higher temperatures (100°, 105° F.) which completely inactivated the organism on both agar and suscept, proved detrimental to the natural defense processes of the corm. Thus the fact that wound periderm was never found in advance of rotted tissue in sufficient amounts to wall off the dead area might have been anticipated. At 95°F. the growth of the organism was not retarded enough for effective wound periderm to form. These results are contrary to a supposition made by Bald (3) that 95°F. checks the growth of *Fusarium* and the corm forms a cork barrier around the injured tissue. *Fusarium*, supposedly, is thus cut off from the inside and prevented from doing further damage. Though this "walling off" has been observed by the writer in a few instances (Fig. 1B) it has always been a natural occurrence and could not be duplicated experimentally.

Of 1120 corms used in the pre-storage tests only 129 from all treatments remained healthy after 2 months' storage. However, these values do not correctly portray the differences between heat and fungicidal treat-

ments. Despite the fact that there was a high percentage of rot in all cases, corms receiving heat treatment with or without accompanying high relative humidity (with the exception of treatment 4A) at least showed an ability to limit spread of the rot. In this respect, heat treatments were far superior to the fungicide treatments.

In general, results of the pre-storage tests indicate 1) that rapid drying at high temperature aids in preventing infection by *Fusarium* and 2) that the high humidity during the curing period is not only nonessential but may be quite undesirable (treatment 4A). Only in treatments 4B and 4C did a high humidity appear beneficial and in both cases corms were not inoculated until after removal from the moist chamber. This is of particular note since in practice the situation would be more like treatment 4A than 4B and 4C, i.e., nearly all natural "inoculation" would occur *before* curing.

The method used is subject to criticism as the corms were not only dipped in a concentrated suspension of active spores, but were also stored at a temperature 20°–30° higher than that recommended for the best control of Fusarium corm rot. These extremes, of course, were designed to test severely the treatments used. Such conditions would never be duplicated in commercial practice and probably the cork layer would never be required to withstand such a severe test under commercial conditions.

SUMMARY

The effect of favorable conditions for wound periderm formation on susceptibility of cut surfaces of gladiolus corms to penetration by *Fusarium oxysporum* f. *gladioli* was tested. The effectiveness of wound periderm in inhibiting penetration by *Fusarium* was clearly evident. Considerable rotting occurred in corms inoculated after 1–4 days. *Fusarium* failed to penetrate those corms inoculated after 5, 6, and 7 days' exposure to conditions favorable for wound periderm formation.

Pre-storage heat treatments with and without an accompanying high relative humidity were tested and their effectiveness in protecting corms from Fusarium rot was compared with 2 recommended fungicidal treatments. Despite the fact that a high percentage of rot occurred in all treatments, corms receiving a heat treatment prior to storage were much less severely rotted than were those treated with either of the fungicides. Rapid mummification of those corms treated with fungicides suggests latent infections which became manifest when the corms were weakened by chemical injury. In all treatments the longer the corms were allowed to cure before cleaning (up to 30 days, the maximum of the experiment), the less severe was the rot. Bruised corms genrally rotted more quickly than did those corms not bruised, but the difference between the 2 groups was slight.

"Walling off" of Fusarium infected areas by a cork barrier, a process presumed to occur in infected corms cured at 95°F., was not observed in this experiment in which the relative humidity was maintained at 95 per cent.

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CIRCULAR SPOT, A DISEASE OF SWEET POTATO ROOTS[1]

W. J. Martin

Roots of the Porto Rico variety of sweet potato in Louisiana occasionally are affected with brown, circular lesions (Fig. 1), which lower the commercial grade of the roots. Although the disease has been severe in some isolated cases, it is considered a minor disease because the losses have been comparatively small. Growers and shippers commonly call the disease circular spot.

For years circular spot symptoms were thought to be associated with soil rot caused by *Streptomyces ipomoea* (Person and Martin) Waksman and Henrici. However, in 1940 Person and Martin described and illustrated symptoms similar to circular spot, stating that such lesions were not produced as a result of their artificial inoculation of sweet potatoes with *S. ipomoea* (4). They also called attention to the similarity of such lesions to some of Halstead's illustrations of soil rot (1).

The purpose of this paper is to report results of more recent investigations establishing the causal agent and distinctive characteristics of the circular spot of sweet potatoes.

SYMPTOMS.—Plants with severely affected roots do not exhibit above-ground symptoms. On the roots the smaller lesions are sunken and the affected tissue cracked. Upon drying, the diseased tissue shrinks and cracks to a greater extent. In some cases the dry tissue in a lesion may be removed, exposing a layer of cork tissue. Occasionally, lesions are large and deep (Fig. 1, B). However, the average lesion is about 1 cm. in diameter. Lesions do not enlarge appreciably after the potatoes are harvested. Rarely, lesions are found which exhibit a fan-shaped mycelial growth, or sclerotia of the fungus *Sclerotium rolfsii*. When lesions are removed before cooking, affected roots are edible.

During the past 5 years circular spot has been observed in many fields where soil rot was not evident.

EXPERIMENTAL WORK AND RESULTS.—Many attempts were made to isolate the causal agent of circular spot by planting pieces of affected tissue on agar plates. Organisms most frequently isolated were bacteria, which were nonpathogenic to sweet potato when used in inoculation experiments. Isolates of *Fusarium* also were obtained, but they failed to cause infection when tested on sweet potatoes. *Sclerotium rolfsii* was isolated from comparatively few of the tissue pieces. This fungus was obtained from relatively new lesions when tissue plantings were made a short time after harvest of the potatoes. In most cases where lesions were invaded by bacteria, *S. rolfsii* was not obtained from tissue plantings.

To test the pathogenicity of *S. rolfsii* to the Porto Rico variety of sweet potato, the following experiment was made. The fungus was allowed to grow on sterilized oats for 10 days. Fifty gm. of this oat inoculum was added to each of 4 12-in. clay pots containing sterilized, sandy-loam soil. The inoculum was mixed thoroughly with the soil and a sweet potato cutting was planted in each pot. Cuttings were planted in 4 pots containing sterilized soil, to serve as controls. The plants were allowed to grow in the pots for 7 months, during which time large roots formed. Roots

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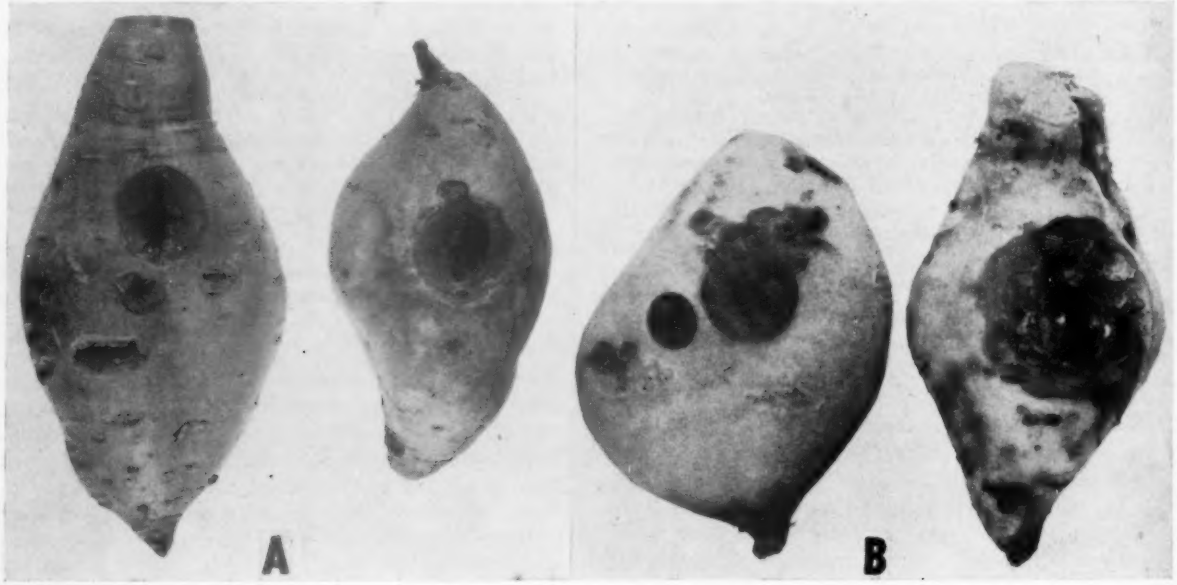

FIG. 1. Sweet potatoes of the variety Porto Rico affected with circular spot. A) Naturally-infected. B) Artificially infected.

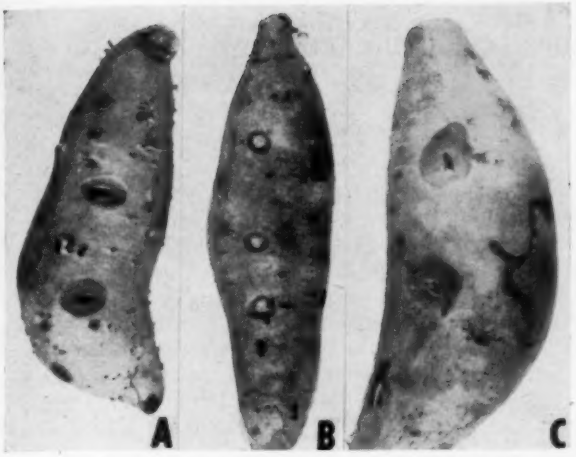

FIG. 2. Sweet potatoes with lesions caused by A) the surface-rot *Fusarium;* B) *Streptomyces ipomoea;* and C) *Pythium* sp.

from the pots containing *S. rolfsii* had typical circular spot lesions; 2 of these roots are illustrated in Figure 1, B. Roots from the controls were smooth and free from lesions. This experiment showed rather conclusively that *S. rolfsii* was capable of causing circular spot lesions.

During investigations on the causal agent of circular spot, experiments were made in which sweet potato roots were inoculated with various fungi which are known to cause diseases of sweet potato. These fungi included *Streptomyces ipomoea, Fusarium* sp., which causes surface rot, and *Pythium* sp., which causes mottle necrosis. Inoculations were made by inserting mycelium into wounds made in the newly-harvested roots kept under moist conditions. Lesions caused by these fungi are illustrated in Figure 2. None of the lesions shrank or cracked upon drying, as is characteristic of lesions caused by *S. rolfsii.* Lesions caused by the surface-rot *Fusarium* continued to enlarge in storage, as recently described (3). Lesions induced by *Pythium* also continued to enlarge when kept under extremely wet conditions. In artificially-inoculated roots as well as in naturally-infected roots, soil rot lesions occasionally resembled circular spot lesions. However, the 2 types of lesions could be differentiated, largely on the basis of the shrinking and cracking upon drying, a characteristic of the affected tissue of circular spot.

Harter and Weimer described a sclerotial blight of sweet potato plants, particularly prevalent in plant beds, and attributed the disease to *Sclerotium rolfsii* (2). While this disease is quite prevalent in plant beds in Louisiana, there appears to be little or no correlation between the sclerotial blight of plants and the circular spot of fleshy roots. The latter appears to be most prevalent in areas where sweet potatoes follow interplanted corn and soybeans, and the inoculum of the circular spot seems to be largely soil borne.

A possible field control measure for circular spot was indicated in 2 field experiments in Louisiana. Although the experiments were designed to study the effect of soil fumigation on the development of stem rot of sweet potato, a considerable amount of circular spot was present in the experimental plots. In 1 experiment in 1950, DD-mixture was used at the rate of 15 gal. per acre before planting. At harvest time a trace of circular spot was evident in each of 4 untreated plots, while none was present in 4 treated plots. In 1952 ethylene dibromide was used at the rate of 2 gal. of Dowfume W-85 per acre. In the latter experiment the average amount of circular spot-affected sweet potatoes in lbs. per acre was 155 for the treated plots and 645 for the untreated plots.

Since 1948 5 field experiments were made using either DD-mixture or ethylene dibromide in soils heavily infested with *Streptomyces ipomoea.* In no case did soil fumigation reduce the prevalence of soil rot in these experiments.

SUMMARY

A disease of sweet potato roots in Louisiana called circular spot is described and attributed to the fungus *Sclerotium rolfsii.* The disease was demonstrated as being distinct from other diseases of sweet potato caused by *Streptomyces ipomoea, Fusarium* sp. and *Pythium* sp. Possible field control measures for circular spot were indicated as a result of soil fumigation experiments with DD-mixture and ethylene dibromide. Soil rot was not reduced in soil fumigation experiments with these chemicals.

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THE STING NEMATODE BELONOLAIMUS GRACILIS STEINER: A PARASITE ON COTTON AND OTHER CROPS IN SOUTH CAROLINA [1]

T. W. Graham and Q. L. Holdeman [2]

The diversity and importance of nematodes as parasites on the roots of crop plants is now generally recognized. In addition to those which penetrate and feed internally, as do the root knot nematodes, *Meloidogyne* spp. and the meadow nematodes, *Pratylenchus* spp., there is a group of surface feeders, sometimes called ectoparasites. Recently the pathological effects of several surface-feeding nematodes have been described by various workers (1), (2), (3), (5), (14). The nematodes involved include the stubby root nematode, *Trichodorus* sp.; the awl nematode, *Dolichodorous heterocephalus* Cobb; the sting nematode, *Belonolaimus gracilis* Steiner; a dagger nematode, *Xiphinema americanum* Cobb; *Tylenchorhynchus claytoni* Steiner; spiral nematodes, *Heliocotylenchus* spp.; and ring nematodes, *Criconemoides* spp.

During the past 3 years increasing evidence has been accumulated of the importance of ectoparasitic nematodes in South Carolina. Primary attention was given to tobacco, cotton and other agricultural crops. An earlier report by the senior writer (8) indicated that *T. claytoni* is an important parasite on tobacco, *Helicotylenchus* spp. on cotton, tobacco and tomato, and *B. gracilis* on cotton and other crops. The particular importance of the sting nematode was pointed out by Christie et al (5) and Brooks and Christie (1) in Florida, by Owens (12) and Miller (11) in Virginia, and by Graham (8) in South Carolina.

A preliminary report has been made (10) on the association of the sting nematode with increased severity of cotton wilt. The present report presents results of recent studies of the sting nematode as a parasite on cotton and other crops in South Carolina.

METHODS.—The modified Baermann funnel technique of Christie and Perry (4) was used to examine soil samples. A standardized procedure was adopted to get uniform, quantitative data on nematode populations from soil collections. Briefly this procedure was as follows: soil samples of 300 cc. were taken from the immediate root area, usually 3–7 in. deep, by means of a ½ in. soil-sampling tube. A composite of 12–15 borings made up a single sample. In the laboratory water was added to the soil collection in amounts of 3 or 4 times the volume of the soil. The mixture was then agitated to separate soil particles and to suspend nematodes in the water. Within a few seconds the larger soil particles settled to the bottom of the container; water and suspended nematodes were poured onto a 20-mesh and then a 200-mesh screen. This washing procedure was repeated 3 times. The material caught on the 200 mesh screen was washed into a 6-in. glass funnel fitted with a muslin filter to catch fine soil particles and other debris washed from the screen. Nematodes worked their way through the muslin and settled to the bottom of the funnel where they were drawn off in clear water after 12–24 hours. Twenty ml. of water was drawn from the funnel into a 90 mm. petri dish. Nematode populations were count-

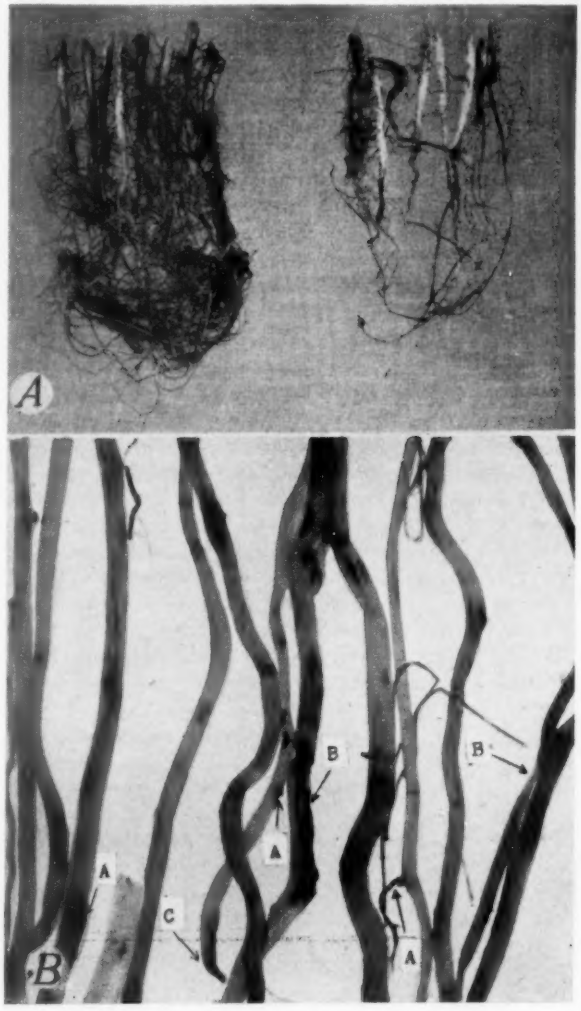

FIG. 1. A) Cotton roots showing effects of sting nematodes on plants grown in greenhouse soil culture. The discolored and sparse roots of infected plants at right are characteristic. B) Root lesions of sting nematodes on cotton. (A arrows) Points where secondary roots were broken off. (B arrows) Discolored and shrunken lesions. (C arrows) Shriveled root tip.

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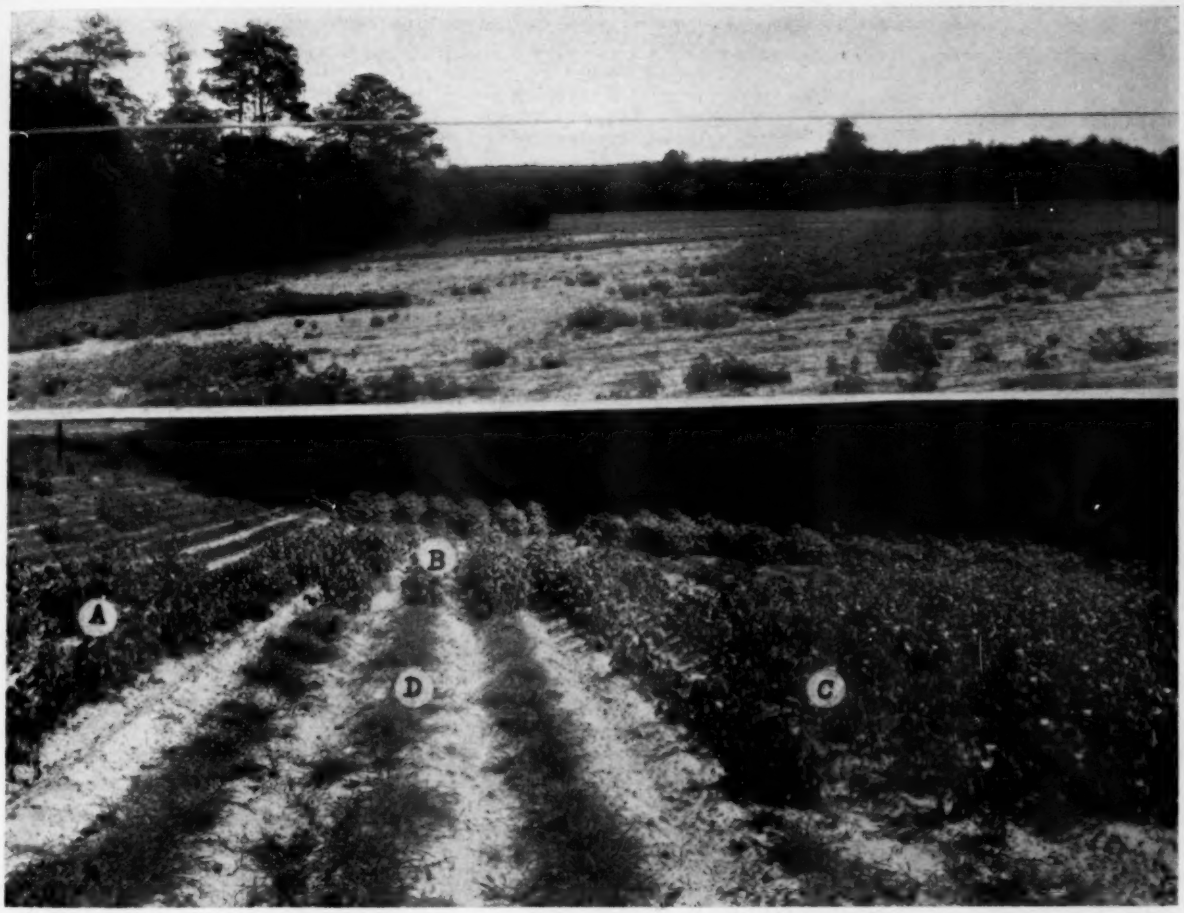

FIGS. 2 and 4. Fig. 2. Cotton field infested with sting nematodes. Infested areas shown here were almost bare of cotton, suffering an estimated over all loss of 50 per cent in 1952. This field has shown a gradual increase in size of infested areas.—Fig. 4. Resulting growth of cotton on fumigated and non-fumigated plots in a field heavily infested with sting nematodes. Plots A, B, and C fumigated with Dowfume W-40 at rates of 10-20 gals. per acre. D, untreated plot. Photographed July 16, 1952.

ed under a stereoscopic microscope. The number of nematodes found in 80 fields (72 × magnification) was recorded as a sample count from each petri dish. This sample was about 5 per cent of the total area of the petri dish, and this served as a basis from which total populations could be calculated.

FIELD OCCURRENCE.—The sting nematode was first found in South Carolina in 1950 in screenings from soil around strawberries and corn, and in 1951 around cotton. Subsequently crop damage in South Carolina by the sting nematode has been found in 7 counties and on some 25 different farms. Screenings gave large numbers of sting nematodes from soil around corn, soybean, cowpeas, cotton, strawberries and sweet potatoes, where these crops were severely stunted and had damaged roots.

The number of crops affected and frequency of damage suggest that more extensive survey would show a more widespread occurrence and damage. It is of particular interest that the sting nematode has not been found in soil collections from tobacco although it has been searched for repeatedly. Tobacco roots were not damaged in soil heavily infested with sting nematodes, and inoculation trials failed to show root damage or increase in nematode population.

SYMPTOMS.—Cotton roots attacked by sting nematodes were severely damaged: in advanced stages roots were destroyed. Careful observation of plants grown in infested soil in early stages of attack showed minute, dark, shrunken lesions along the root axis or at the root tip. Lesions sometimes advanced laterally to girdle the roots, which soon broke off, and in other cases advanced lengthwise and persisted for some time without severing the roots. The latter case appeared to be the result of repeated attacks along the same root. These shrunken discolored roots gave the entire root system of affected plants a general unhealthy appearance (Fig. 1, A). Fig. 1, B illustrates the shrunken and discolored lesions observed in the early stages. Proliferation of new roots above the point of attack, or "stubby root" symptoms, was not observed in cotton but was sometimes pronounced in corn. Affected soy-

TABLE 1.—*Body measurements of sting nematodes from cotton soil*

Parts measured	Female				Male			
	Length		Body Width		Length		Body Width	
	Range	Average	Range	Average	Range	Average	Range	Average
	Microns							
Front of Head to:	10.6-	11.5[a]						
Base of head	11.9		19.9-23.9	22.0	10.6-11.9	11.1	17.2-19.9	18.2
Base of stylet	114.3-133.0	126.3	33.2-46.5	40.2	115.7-129.0	119.3	30.5-41.2	32.7
Nerve ring	182.2-212.8	203.4	37.2-53.2	46.5	186.2-202.1	194.9	34.5-46.5	37.7
Base of esophagus	312.0-321.0	316.0	53.2-58.5	55.1	284.0-309.0	295.2	39.9-47.8	43.2
Vulva	1064.7-1297.8	1159.8	45.2-63.8	54.9	724.5-[b] 1102.5	964.5	39.9-49.2	43.8
Anus to terminus	127.6-146.3	132.3	34.5-50.5	42.5	121.0-151.6	140.2	23.9-29.2	26.6
Total length [c]	2009.7-2501.1	2230.8	.....	.....	1738.8-2129.4	2018.5	.....	.....
Egg's—Width × Length	33-40 × 127-149	36 × 144						

[a] Averages based on measurement of 6 females and 7 males.
[b] These measurements on males taken at center of the body.
[c] Selected measurements from this table expressed as De Man's proportional formula are as follows:

$$a = \frac{\text{total length}}{\text{body width}} \qquad b = \frac{\text{total length}}{\text{esophagus length}} \qquad c = \frac{\text{total length}}{\text{tail length}}$$

v = position of vulva in relation to body length in percentage

♀ = total length 2230.8μ a = 40.6 b = 7.0 c = 16.8 v = 51%
♂ = total length 2018.5μ a = 46.0 b = 6.8 c = 14.3

bean and cowpea roots had much the same appearance as cotton.

Field symptoms of sting nematode damage in all affected crops were severe stunting frequently accompanied by early death. Infested field areas were variable in size and shape but their margins were usually well marked. Fig. 2 illustrates a cotton field infested with sting nematodes where the crop loss was about 50 per cent. Damage by sting nematodes cannot be identified alone by retarded growth or by damaged roots because these symptoms may be caused by other parasites. Nor can sting nematodes be found by microscopic examination of roots since they are not attached to the roots. Positive diagnosis therefore can only by made by soil examination.

THE CAUSAL ORGANISM.—The original description of the sting nematode was given by Steiner (13) in 1949 who designated it as a new genus, and new species of the family *Tylenchidae*. The type material was collected on roots of slash pine, *Pinus caribaea* Morelet and long leaf pine, *P. palustris* Mill., in Florida. To date this species has been reported only in southeastern United States. It is easily recognized by its unusually long, slender body and long stylet (Fig. 3). Both males and females were numerous in South Carolina collections. Camera lucida drawings and body measurements illustrate detailed characters used to identify this nematode (Table 1).

PATHOGENICITY TESTS.—In preliminary tests sting nematode-infested soil was brought to the greenhouse where the typical symptoms found in the field were reproduced on cotton, corn, soybean, cowpeas, strawberries and sweet potatoes. Sting nematodes collected in a Baermann funnel were placed in sterilized soil; suspects grown in this soil showed typical root injury. Large numbers of sting nematodes were recovered from the soil around the roots of these plants. In a more carefully controlled experiment 100 sting nematodes were picked singly, collected in a few drops of water, and placed in each of 5 6-in. pots of sterilized soil. A similar series of pots without nematodes served as controls. One crop of cotton was grown in these pots for 47 days to allow the nematodes to increase. After counting nematode populations in each pot the soil from all 5 pots was combined and thoroughly mixed to obtain equal distribution of nematodes. The soil was repotted; by calculation each pot then contained approximately 600 nematodes. A second crop of cotton was planted with surface-disinfected seed in both infested and control soils. Soil temperatures were maintained at 85–90°F. After 3 weeks the inoculated plants showed marked growth retardation as compared with the controls. Final data were taken 76 days after planting (Table 2). There was a direct correlation between number of sting nematodes and the amount of plant damage. However, considering the data from individual pots the greatest damage to roots was not necessarily accompanied by highest nematode counts. For example, nematode counts in pots 2 and 4 were 11,200 and 14,400 and respective disease indexes were

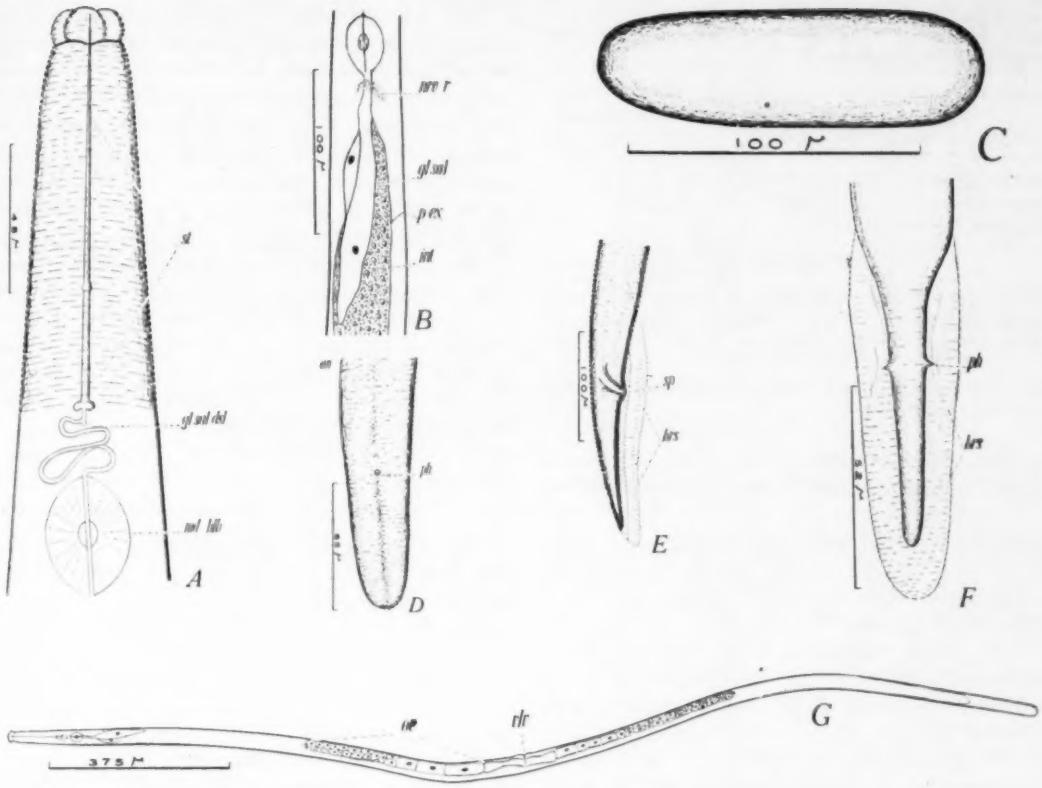

FIG. 3. Camera lucida drawings of the sting nematode, *Belonolaimus gracilis* Steiner. A) Anterior portion of adult female: *st*—stylet; *gl sal dsl*—opening of dorsal salivary gland near base of stylet; *md blb*—median bulb. B) Salivary gland and anterior intestinal region of adult female: *nrv r*—nerve ring; *gl sal*—salivary gland; *p ex*—excretory pore; *int*—intestine. C) Egg. D) Posterior of adult female: *an*—anus; *ph*—phasmid. E and F) Posterior of adult male: *sp*—spicula; *brs*—bursa; *ph*—phasmid. G) Adult female: *ov*—ovary; *vlv*—vulva.

45 and 50, whereas in pots 1 and 3 counts were 5,479 and 3,240, accompanied by disease indexes of 75 and 85. A similar observation was recorded in earlier studies with meadow nematodes parasitizing tobacco and corn roots (7). Highest populations of meadow nematodes were reached in early or midseason growing stages when an abundance of living roots was available as food supply. With continued root decay, and a decreasing food supply, nematode populations decreased. Results with sting nematodes show this same relationship.

A careful search was made for sting nematodes in

TABLE 2.—*Growth of cotton, root injury, and sting nematode populations in greenhouse pot inoculation trials*

Measurements and weights	Sting nematode soil pot number						Control pot number					
	1	2	3	4	5	Ave.	1	2	3	4	5	Ave.
Root weight (grams)[a]	7.0	8.0	5.3	4.2	5.0	5.9*	7.3	6.3	9.3	9.0	9.3	8.2
Top weight (grams)	10.6	11.0	9.7	6.5	10.0	9.5**	12.6	14.6	12.6	16.3	11.6	13.5
Plant height (inches)	11.1	10.5	9.7	9.2	11.0	10.3**	12.6	13.3	12.3	13.0	12.3	12.7
Amount root injury[b]	75	45	85	50	50	61.0	0	0	0	0	0	0
Number sting nematodes[c]	5,479	11,200	3,240	14,400	10,200	8,903	0	0	0	0	0	0

[a] Average green weight per plant, 2 to 4 plants per pot.
[b] Disease index, 0 = no damage, 100 equals all roots dead.
[c] Calculated total for entire pot based on sample counts.
* Difference between plants in infested and control soil lots were statistically significant at the 0.05 percent level.
** Difference between plants in infested and control soil lots were statistically significant at the 0.01 percent level.

TABLE 3.—*Effect of soil fumigation on cotton stands, boll counts, and sting nematode population in field plots fumigated with Dowfume W-40*

	Fumigated plots	Non-fumigated plots
Plant stands (May 21)[a]	68.0	51.8
Boll counts (Aug. 21)	340.0	16.5
Number sting nematodes (Aug. 21)[b]	0	50

[a] Average counts from 4 replicates of 4 rows each; rows 32 ft. long. Difference between fumigated and non-fumigated plots were statistically significant at the .01 per cent level.

[b] Total sting nematodes in a single 300 cc. composite sample of soil from each plot.

roots of cotton but none were found. They were found only in soil which indicated a strict external feeding habit.

CONTROL BY SOIL FUMIGATION.—Greenhouse trials showed that soil fumigation was effective against the sting nematode. Successful soil fumigation in field treatments were reported by Christie et al (6), and Miller (11). Field fumigation trials with row treatment were conducted at several locations where cotton land had shown declining yields over a period of years until it was practically worthless for cotton production. The results showed striking differences between the normal vigorous plants in fumigated soil and the usual stunted, irregular stands in untreated soil. Experimental plots were treated on a grower's farm in 1952, in a field with a history of almost complete failure of cotton for several years and known to be heavily infested with sting nematodes. Results in Table 3 show a marked stand improvement in fumigated soil when readings were taken on May 21. On August 21 a very striking difference in the number of surviving and fruiting plants was observed. The cotton in the untreated plots made practically no growth (Fig. 4).

TABLE 4.—*Number of sting nematodes and meadow nematodes following greenhouse soil fumigation tests with Dowfume W-40*[a]

Nematodes	Control not fumigated	Amounts of Dowfume W-40 used per 1-gal. crock		
		0.25 ml.	0.5 ml.	1.0 ml.
Number of meadow nematodes	2568	1080	0	0
Number of sting nematodes	672	0	0	0

[a] Separate lots of soil infested with each nematode were treated with the above dosages which is the equivalent of approximately 20, 40, and 80 gal. per acre of Dowfume W-40. Soil was covered with moisture-proof paper for 8 days after fumigation. Soil temperature was 80–95°F. The Baermann funnel technique was used for washing and screening soil samples. Nematode counts represent the estimated total per crock. Results are averages from 2 tests.

Soil samples taken from the fumigated plots on August 21 were free of sting nematodes (Table 3) indicating that fumigation was more effective against the sting nematode than was reported earlier by Graham and Holdeman (9) in other fumigation experiments to control the meadow nematodes. In 1950, meadow nematode infested soil had been fumigated at the rate of 30 gal. of Dowfume W-40 per acre, but this suppressed meadow nematodes only during the early part of the growing season. Population counts thereafter from mid-July through September were higher than in untreated soil. A further comparison of the effectiveness of fumigation on the sting nematode and the meadow nematode was made and is shown in Table 4. Twenty gal. per acre of Dowfume W-40 eliminated only about ½ the meadow nematodes but this treatment killed all sting nematodes.

SUMMARY AND CONCLUSIONS

The sting nematode, *Belonolaimus gracilis*, has been observed to cause serious losses to cotton, corn, soybeans, and cowpeas in South Carolina. In the case of cotton, large areas were found where infestation was so severe that there was practically no plant growth. Field symptoms are growth retardation and generalized root decay. Such symptoms were reproduced in greenhouse tests. Field symptoms, due to sting nematode damage, are not distinguishable from those due to other causes. Positive identification of the sting nematode disease can be made only by soil examination and identification of the parasite. The usual microscopic examination of diseased roots does not aid diagnosis since no organism is present. The sting nematode is relatively easy to recognize because of its long slender body and long stylet. When infested soil was fumigated with a nematocide normal production was restored. Soil fumigation gave more effective control of the sting nematode than the meadow nematode. Since tobacco is not attacked by the sting nematode, it can be used as a rotation crop with cotton on areas where this nematode is a major problem.

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EFFECT OF ULTRA-VIOLET, X-RAYS, AND NUCLEAR RADIATIONS ON RING-ROT BACTERIA INFESTING BURLAP POTATO BAGS [1]

G. H. Starr and C. A. Cinnamon [2]

It is a matter of common knowledge among potato workers that ring-rot bacteria are carried on reused burlap bags, which often carry the ring-rot organism from one location to another either in picking up potatoes at harvest time, or for storage. The reuse of these bags, infested by diseased tubers, provides ample opportunity for the spread of ring rot through the medium of burlap. If burlap bags could be decontaminated by some practical method, it would be a valuable aid in the control of ring rot.

Research conducted at the Wyoming Agricultural Experiment Station the past few years showed that the causal bacteria can live on burlap bags up to 5 months in a storage cellar and as much as 3 months outside.[3] Subsequent tests showed that burlap bags tied in bundles harbor live bacteria for as much as 7 months inside a storage cellar.[4] In addition, burlap bags—hung singly so that 1 side was exposed to direct sunlight—carry few viable bacteria after 40 days and none after 75 days. Further research showed that steam sterilization is effective in killing ring-rot bacteria on burlap bags, although the bags are damaged somewhat in appearance in the process.[5] The use of dry heat, although effective, was found to be more time-consuming than expected.[3]

Since ultra-violet lamps are easily available, they could be adapted for disinfestation if they should prove effective in killing ring-rot bacteria in experimental trials. The purpose of this experiment was to test the effectiveness of ultra-violet light, x-rays and nuclear radiations.

EXPERIMENTAL PROCEDURE.—Circles of burlap, approximately 100 mm. in diameter, were contaminated with ring-rot bacteria (*Corynebacterium sepedonicum*) from heavily infected Bliss Triumph tubers. In the process of contamination, the freshly-cut, infected tubers were rubbed over the surface of the burlap 12 times in a back-and-forth motion. Two infected tubers were used in such manner for each burlap piece contaminated. These same 2 tubers were used to contaminate all pieces of burlap. The circles of burlap were left in the laboratory for 2 days before exposure to the various types of radiation.

A Glo-Craft mercury vapor lamp produced the ultra-violet radiation with a beam of radiation approximately 10 in. in diameter. The burlap pieces were mounted as described 22.5 cm. from the face of the lamp and in the center of the beam. During the interim of irradiation, temperature of samples rose to 43°C. Intensity of the beam was measured in arbitrary units on a Photovolt Corporation instrument with a 0.24 mm. diameter aperture over the photo cell. Intensity of the u v radiation gave a reading of 70 divisions on the high scale. A test for uniformity of radiation indicated a variation in intensity of ± 5 per cent.

X-rays used were "unfiltered" general radiation of 2 distinctive energy ranges, identified as "High Voltage X-rays" and "Low Voltage X-rays." A 150 K V Thermax tube, operated on a 150 K V Industrial Westinghouse unit, produced the former. The tube was self-rectified and contained in an oil-filled, water-cooled housing. Operating conditions for the various times of exposure given in Table 1 were as follows: 140 KV, 5 ma. at 36 cm. focal-object distance and sample temperature 30°C. A radiator bifocal tube, R B 1-4 (General Electric), operated under full wave mechanical rectification at 50 K V P and 10 - 12 ma., produced the Low Voltage X-rays. Samples were placed at a focal-object distance of 25 cm. and attained a temperature of 28°C during irradiation.

Dosage in roentgens was measured by a Victoreen Condenser R-meter. The observed readings were corrected for temperature and barometric pressure to

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TABLE 1.—*Effectiveness of different rays in killing ring-rot bacteria contaminating burlap bags, as shown by potato plants as indicators*

Treatment with	Length of Exposure	Measured Dosage in Roentgens reduced to S T P	Percentage of Infection	Severity of Infection
Ultra violet	5 min.	---	100	3.1
Ultra violet	15 min.	---	100	2.7
Ultra violet	30 min.	---	90	3.2
Ultra violet	60 min.	---	100	4.2
High voltage X-rays	1 min.	75	100	4.3
High voltage X-rays	5 min.	380	100	4.6
High voltage X-rays	10 min.	880	100	4.4
High voltage X-rays	15 min.	1175	100	4.4
Low voltage X-rays	1 min.	160	90	3.7
Low voltage X-rays	2½ min.	425	90	3.8
Low voltage X-rays	5 min.	850	100	4.6
Low voltage X-rays	10 min.	1680	70	3.5
Radio-Phosphorus 32 (beta particle radiation)	23.5 hr.	.26	100	4.2
Radio-Iron 59 (beta & gamma radiation)	22 hr.	4.8	78	2.8
Radio-Zinc 65 (beta & gamma)	22 hr.	25.0	100	3.6
Radio-Iodine 131 (beta & gamma)	23.5 hr.	2.3	100	4.0
Radio-Cobalt 60 (beta & gamma)	22 hr.	4000	89	3.3

give the roentgen equivalent at standard temperature and pressure (S T P). The corrected value appears in Table 1.

Intensity of the nuclear radiation from the various radioisotopes was measured at the position occupied by the sample under test with a "Juno" type of dose-rate ionization chamber. The instrument was calibrated for beta-gamma radiation against radium to approximately ± 15 per cent.

The contaminated burlap pieces were placed in a position perpendicular to the direction of the radiation and at a known distance from the source. The various rays were used for predetermined periods of time, after which the treated burlap pieces were placed in sterile envelopes with sterile forceps. All series were duplicated. Tests were completed in 2 days, June 2 and 3, 1951. The following day all treated burlap pieces were placed in sterile petri dishes, 1 to a dish. Sterile water was poured over each piece to liberate the ring-rot bacteria.

To determine whether the bacteria had been killed, 5 healthy potato seed pieces were placed in the bacterial suspension in each dish. Each seed piece was freshly cut through a potato eye so that this eye was in contact with the bacterial suspension for a period of 60 minutes. Seed pieces were then removed with sterile forceps, placed in sterile paper bags, and planted at the Agronomy Farm near Laramie. The 5 seed pieces of each treatment lot were planted in units 18 in. apart in the row.

These units were inspected several times during the growing season for the development of ring-rot symptoms in the plant. Final reading was made on September 7, with an inspection of each plant for ring-rot symptoms. If symptoms were present, degree of severity was recorded, using a scale of 0-5 as follows, in terms of ring-rot symptoms: 0) none; 1) slight, but definite; 2) approximately ¼ of plant infected; 3) approximately ½ of plant infected; 4) approximately ¾ of plant infected; and 5) total infection or death of plant.

The disease-severity readings reflected the efficiency of the ray treatments. (Readings of zero or low indicate that rays were effective in killing bacteria on burlap; high readings indicate rays were not effective.) Table 1 shows pertinent data for this test.

SUMMARY AND CONCLUSIONS

Ultra-violet rays, X-rays and nuclear radiations were used experimentally to kill ring-rot bacteria (*Corynebacterium sepedonicum*) infesting burlap potato bags. Exposures of ultra-violet rays up to 60 minutes, high voltage X-rays up to 15 minutes, low voltage X-rays up to 10 minutes, and nuclear radiations of 22 hours or more, were not effective in killing ring-rot bacteria; neither did they indicate practical value in ring-rot control. On the materials used, however, the radioactive iron gave some evidence of partial control and possibly should be tested further.

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THE GROWTH OF THE OAK WILT FUNGUS WITH VARIOUS VITAMINS AND CARBON AND NITROGEN SOURCES [1]

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A knowledge of the basic nutrient requirements of *Endoconidiophora fagacearum* Bretz (*Chalara quercina* Henry) would be helpful in dealing with many aspects of the oak wilt problem. For example, the use of relatively simple nutrients with known chemical formulae could place studies of toxic metabolites on a sounder footing. Investigations of host-parasite relationships and of longevity of the organism under various conditions, as well as isolation from suspect vectors could be furthered by media which favor *Chalara* above associated organisms.

Results of nutritional studies with *Chalara* have been reported by Hoffman (6), and summarized in abstracts by Barnett and Lilly (1), and White and Wolf (10). Young (11) studied the physiology of this organism in relation to production and germination of conidia. Some differences in results have appeared. Experimental conditions may account for some of these differences although extensive details of methods could not be given in abstracts. Variability in nutrient requirements among isolates also may account for differences in results.

The purpose of this study was to investigate the effect of various carbon and nitrogen sources and various vitamins on the growth of *Chalara quercina*. The results obtained were examined for clues concerning the mechanism of wilt development.

MATERIALS AND METHODS. — All glassware was cleaned in a chromate-sulfuric acid solution followed by 10 hot tap-water rinses. No effect of chromate residues could be detected. In vitamin studies, glassware was prepared in this manner before each experiment. Otherwise the acid soak was eliminated between experiments. Six-oz. prescription bottles were used as culture vessels. Usually, screw caps were used because they proved more convenient than, and allowed as much growth as, cotton plugs. Pyrex wool stoppers were used in vitamin studies to reduce the possibility of biotin contamination.

Liquid media were used throughout. These were prepared with reagent grade chemicals and distilled water except in certain of the early physical-chemical studies. Generally, 25 ml. aliquots were used for physical-chemical and vitamin studies while 10 ml. were used in nitrogen and carbon experiments. Acidity was measured with a Beckman pH meter and adjusted with NaOH or HCl. Unless otherwise specified, media were autoclaved for 20 minutes at 15 lbs. pressure.

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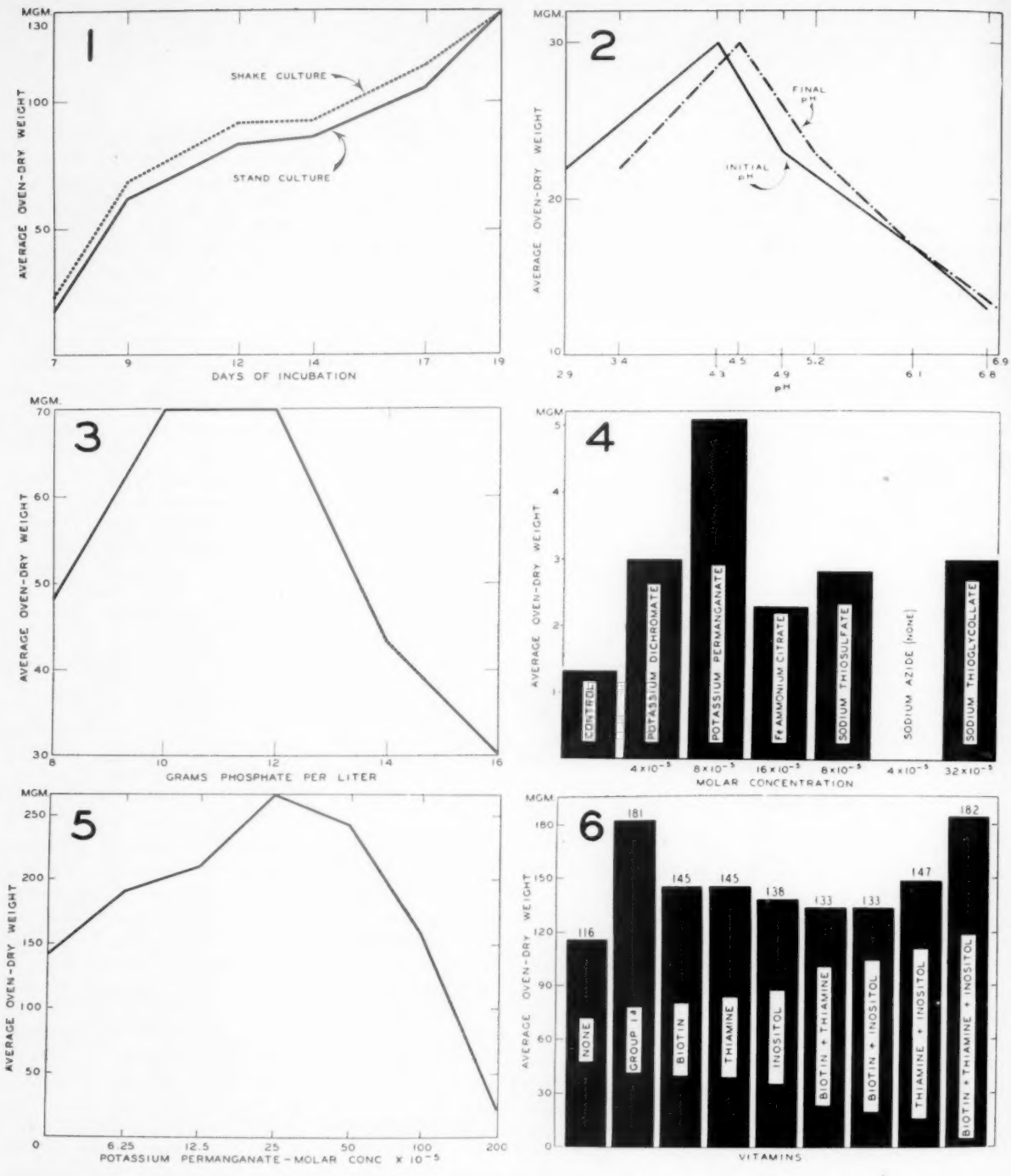
The culture used was obtained from a wilting red oak in Wisconsin. Inoculum was prepared from subcultures of a single-conidium isolate. These cultures were grown for 2–3 weeks on potato-dextrose agar. In vitamin studies conidia for seeding media were washed 3 times with distilled water by centrifugation. Seeding of test media was accomplished by pipetting given volumes of standardized spore suspensions. No differences in growth could be detected with a spore load of 5,000 conidia or more per ml. of medium. Ordinarily a minimum level of 7,500 conidia per ml. of culture medium was established. However, in vitamin studies as few as 130 conidia per ml. of medium were employed to reduce any residues carried by the conidia.

A suitable incubation period was sought. Culture at 26°C. on several good nutrient media showed little growth before 6 days, rapid growth through 12 days and a period of somewhat slower growth through 30 days (Fig. 1). Unless otherwise specified an incubation period of 14 days was used. The temperature of 26°C. was selected on the basis of earlier work by Henry (5) and Young (11).

Growth was recorded as the oven-dry weight of the mycelium. The liquid media were decanted from the mycelial mats. These were allowed to soak in 100 ml. of distilled water for 2 hours. The excess water was drained away through wire gauze cones in funnels. The mats were placed in aluminum weighing cups and were dried at 95°C. to constant weight, usually about 3 hours.

Five to 7 aliquots of each medium were employed in each experiment. Generally, each experiment was repeated 3 times. In certain designs in which several concentrations served as reciprocal checks these were repeated only twice. In vitamin studies in which differences were small and sometimes questionable, experiments were repeated as many as 6 times.

EXPERIMENTAL RESULTS.—*Physical-chemical conditions.*—The effect of aeration was tested on a buffered, sucrose-yeast extract medium. In quiescent culture, bottles were placed on a side to increase the surface area while in the aerated series bottles were placed vertically in a reciprocating shaker having 52 4-in. cycles per minute. Little difference in dry weight yield resulted (Fig. 1). A good mat formation occurred in stand cultures while a more yeast-like growth developed in the shake series. Some variation in the amount of growth was apparent in stand cultures early in the incubation period. This apparently resulted from differences in the time of surfacing of the mycelium. However, these differences were not pronounced after 14 days of incubation. Preparation and weighing manipulations were considerably more rapid and loss tended to be less with the mat-type growth. Qui-



FIGS. 1–6. Fig. 1. Average oven-dry weight of aerated and non-aerated cultures in relation to the incubation period.—Fig. 2. The average oven-dry weights of mycelium from 9-day-old cultures buffered at various pH levels with 10 gm. of phosphate.—Fig. 3. The average oven-dry weight of mycelium from 10-day-old cultures containing various levels of phosphate buffer. The initial pH was 5.—Fig. 4. The average oven-dry weight of mycelium produced on media to which oxidizing and reducing agents were added. The concentration indicated for each material is that which supported best growth.—Fig. 5. The average oven-dry weight of mycelium produced on media containing various concentrations of potassium permanganate.—Fig. 6. The average oven-dry weight of mycelium from cultures grown on 25 mls. of medium containing three vitamins singly and in various combinations. Incubation was for 14 days at 26°C. a. In group Ia, riboflavin was omitted because of apparent inhibition.

escent culture as described above was therefore adopted as the standard method.

The pH range which best supported growth was determined by varying the proportions of mono-, di-, and tri-hydrogen phosphate of potassium. Early results showed that considerable acid was produced at first. This was followed by a gradual increase in alkalinity. A total of 10 gm. of phosphate per liter was used to maintain the pH at a relatively uniform level. Best growth was obtained between 4.0 and 5.0 (Fig. 2). An initial pH of approximately 4.5 was adopted for subsequent experiments.

A need for considerable buffering was apparent. In media to which 1½ gm. of phosphate per liter had been added, the pH dropped rapidly from 5 to 3 or below. Growth at this level was poor. Mono- and di- potassium phosphates were added at a ratio of 17:1 to give a pH of approximately 5. The total concentration of phosphate was varied to give 4, 8, 16, and 32 gm. per liter. Maximum growth was obtained at 8 and 16 gm. per liter. In subsequent experiments, best growth occurred at 10 and 12 gm. per liter (Fig. 3). Unless otherwise specified, 12 gm. per liter of medium were used in subsequent experiments. Under these conditions the pH remained relatively constant (Fig. 2).

The effect of various oxidizing and reducing agents upon growth was determined. Six chemicals were employed, each at 7 concentrations. Because of the large number of containers necessary, culture tubes were used in place of bottles. A total of 5 ml. of a sucrose-yeast extract medium was added to each tube. After sterilization and cooling, the oxidizing and reducing agents were added aseptically. The average oven-dry weight of mycelium at the best concentration of each material is given (Fig. 4). Two interesting results were observed. A 2-fold increase in growth was obtained with potassium permanganate at 8×10^{-5} molar concentration. Similar results were obtained with potassium permanganate in further tests using the bottle culture method (Fig. 5). In these experiments best growth was obtained at 25×10^{-5} molar concentration. On the other hand, sodium azide prevented growth even at the lowest concentration (4×10^{-5} molar). However, potassium permanganate was not used in subsequent nutritional studies because of the chemical changes which might have been induced in these qualitative experiments.

Since most studies were to be made on synthetic media, minor elements were tested. Various levels of the Robbins and Ma (7) solution were added to a sucrose-asparagine-salts medium. Evidence of a deficiency appeared when none were added. The condition was corrected by the addition of 1 ml. of this solution per liter of medium.

Vitamin requirements.—Sixteen vitamins and purines (Table 1) were tested for activity in promoting growth of *Chalara* on a buffered sucrose-"vitamin free" casein hydrolysate medium. Five 25 ml. aliquots were used in each replicate. In the first series of experiments each of 3 groups of vitamins was omitted from 1 of 3 treatments. Two additional check treatments contained no growth substances and a full complement of all 16, respectively. An incubation period of 8 days was used in this experiment to facilitate screening. In subsequent experiments the standard 14-days incubation period was used. The average oven-dry weight of mycelium produced in the medium containing all vitamins in groups I, II, and III was 136 mg. Relatively poor growth [30 mg.] occurred in the no vitamin treatment and in that from which group I had been omitted [45 mg.]. Relatively good growth appeared in the treatments from which groups II [103 mg.] and III [108 mg.] had been omitted. These results showed *Chalara* to be at least partially deficient for 1 or more growth substances. The most active of these were in group I.

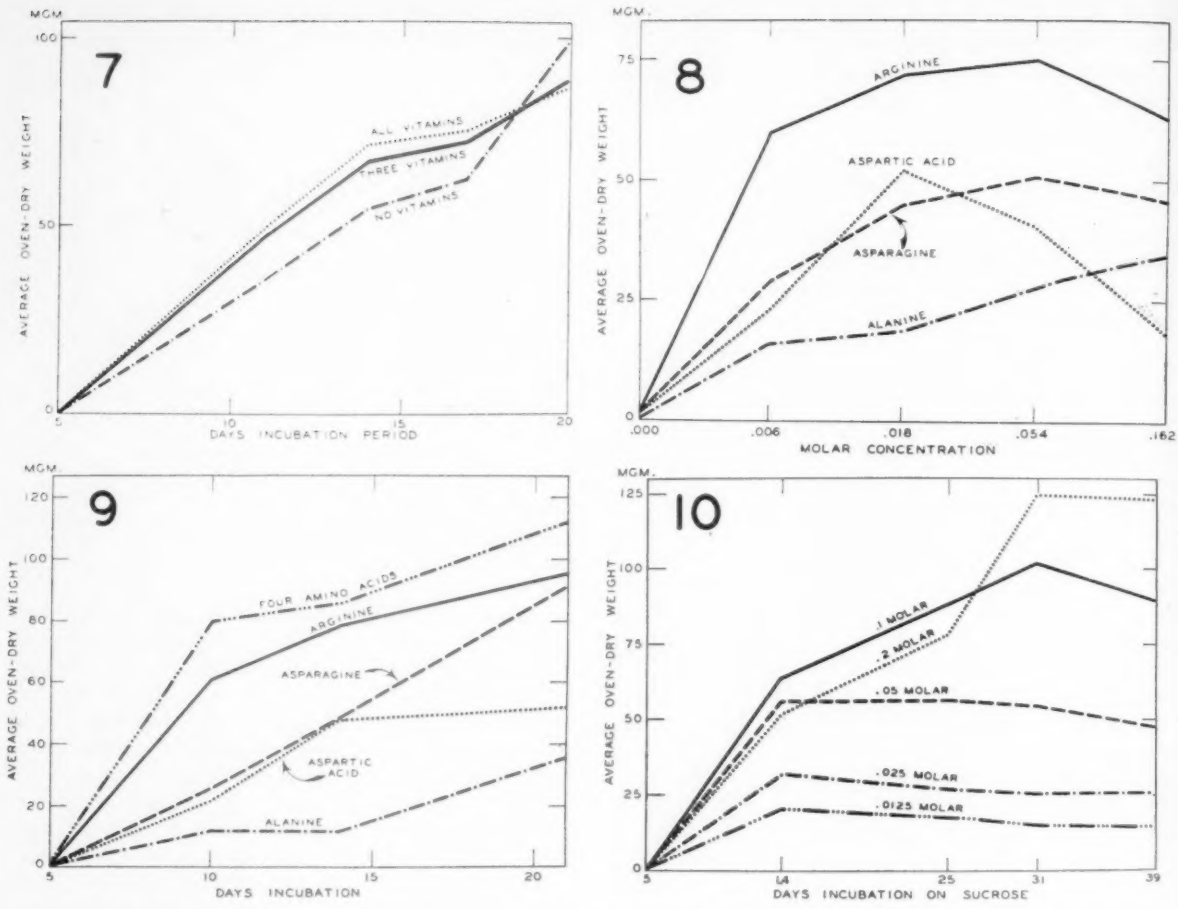
TABLE 1. *Vitamin and purine sources and concentrations*

	Vitamin	Concentration mg. per liter
Group I	biotin	.025
	i-inositol	50.0
	nicotinic acid	.5
	pyridoxine	1.0
	riboflavin	1.0
	thiamine hydrochloride [a]	1.0
Group II	p-amino benzoic acid	.5
	l-ascorbic acid	.5
	calcium pantothenate	1.0
	choline chloride	20.0
	pimelic acid	.5
	Purine	
Group III	adenine sulfate	.5
	glutamine	.5
	guanine	.5
	uracil	.5
	xanthine	.5

[a] Thiamine was prepared in 70 per cent ethyl alcohol and added aseptically to the autoclaved medium. All other compounds were added to the medium before autoclaving.

To determine which of the 6 vitamins in group I promoted growth, all but 1 were added to each of 6 treatments. No vitamins and all those in group I were included as check treatments. This experiment was repeated 6 times. Although the total growth varied, media from which biotin, thiamine, or inositol were omitted consistently supported less growth than the complete medium. Those from which nicotinic acid or pyridoxine, respectively, were omitted consistently supported as good growth as the complete medium, while that from which riboflavin was omitted supported better growth than the complete medium. Riboflavin was therefore eliminated from group I in subsequent experiments and the group was designated Ia.

The 3 vitamins which showed beneficial effects were tested in all possible combinations. The maximum yield in media containing single vitamins or any combination of 2 vitamins was 80 per cent of that in the complete medium. However, media supplied with all 3 produced a level of growth equal to that containing all group I vitamins (Fig. 6).



FIGS. 7–10. Fig. 7. The average oven-dry weight of mycelium produced on 10 mls. of medium containing no vitamins, 3 vitamins, and all 16 vitamins after various incubation periods.—Fig. 8. The average oven-dry weight of mycelium produced on the 4 best nitrogen sources.—Fig. 9. The average oven-dry weight of mycelium produced after various incubation periods on the 4 best nitrogen sources at .02 molar concentration and a mixture of these each at .005 molar concentration.—Fig. 10. The average oven-dry weights of mycelium produced on media containing various sucrose concentrations harvested after 5, 14, 25, 31 and 39 days of incubation.

The degree of deficiency for these 3 vitamins was determined by measuring the oven-dry weight of mycelium produced during various incubation periods. The basal medium in this experiment was similar to that in earlier trials except that asparagine was substituted for "vitamin-free" casein hydrolysate. The volume of medium per aliquot was reduced from 25 to 10 ml. Growth curves for media to which 3 vitamins or all 16 growth substances had been added were comparable (Fig. 7). An early lag appeared in the no vitamin treatment. Later the rate of growth and total yield attained were comparable to that in the other media. Therefore, the deficiency for these vitamins with this isolate appeared to be relatively unimportant.

Nitrogen sources.—Growth with each of 25 nitrogen sources was determined on 10 ml. aliquots of a buffered sucrose-salts medium. These sources included nitrate and ammonium salts, urea, and amino acids (Table 2). All but the highly insoluble salts were supplied singly at .006, .018, .054 and .162 molar concentrations. Urea was sterilized by filtration and added aseptically.

The best growth was obtained with arginine. Asparagine and aspartic acid supported good growth although aspartic acid became inhibitive at the higher concentrations (Fig. 8). Fair growth resulted with urea, alanine, and ammonium salts while poor growth was obtained with glycine, phenylalanine, threonine, tryptophane, methionine and histidine. In addition, no growth was obtained at any of the above concentrations with nitrate salts, leucine, isoleucine, hydroxyproline, tyrosine, cystine, cysteine and lysine. An .018 molar concentration of most sources supported a level of growth comparable to, or greater than, all other concentrations.

Growth rates and total growth with the best nitrogen sources were determined by measuring the dry weight of random replicates after various incubation periods. A combination of arginine, asparagine, aspartic acid and alanine (each at .005 molar concen-

TABLE 2. *Dry weight yield at the best concentration of various nitrogen sources*

Compound	Source[a]	Grade[b]	Yield[c]	Molarity[d]
NH_4NO_3	Merck	R	22	.018
NH_4Cl	Mall.	—	18	.018
$(NH_4)_2SO_4$	Baker	—	18	.018
$NH_4H_2PO_4$	Merck	CP	22	.018
$(NH_4)_2HPO_4$	Merck	CP	18	.018
Urea[e]	—	—	26	.018
Glycine	EK	—	11	.162
dl-alanine	Pfan.	CP	35	.162
dl-phenylalanine	Pfan.	CP	11	.054
dl-threonine	Merck	—	5	.018
dl-tryptophane	Merck	—	6	.006
dl-methionine	Merck	—	12	.018
l+aspartic acid	Mann	CP	52	.018
l+histidine	Lemke	—	10	.018
l+arginine	Merck	—	75	.054
l—asparagine	Mann	—	51	.054

[a] Mall., Mallinckrodt; EK, Eastman Kodak; Pfan., Pfanstiehl.
[b] R, Reagent; CP, Chemically Pure.
[c] Average oven-dry weight in milligrams from best concentration.
[d] Molar concentration supporting best growth.
[e] Sterilization by filtration.

tration) supported a somewhat higher level of growth than arginine alone (Fig. 9). Although the growth rates with asparagine and aspartic acid were similar, asparagine supported a greater total growth. The rate of increase with asparagine was uniform after that with aspartic acid had dropped off.

Carbon sources.—A large number of compounds were to be tested for their value as carbon sources. Therefore, each was supplied at 1 concentration only. Experiments were set up to determine a good standard level. The basal medium contained inorganic salts and asparagine at .054 molar concentration. This concentration was used to insure that nitrogen would be plentiful but not inhibitive. Sucrose was added at .012, .025, .05, .1, and .2 molar concentrations (Fig. 10). The yield increased uniformly through the .1 molar level. Additional carbon allowed no increase in growth during the first 25 days. The .1 molar concentration was selected since it supported a sustained high rate of growth. However, the early rate of growth at the .05 molar level was comparable to that with .1 molar sucrose.

Nineteen saccharides and polysaccharides (Fig. 11) were tested at a carbon level which was equivalent to that in .1 molar sucrose. Nine alcohols and 11 organic acids were tested at a carbon level equivalent to that in .015 molar sucrose which served as a check. This lower concentration was used to reduce the possibility of toxic effects. Sterilization of these compounds, where necessary, was accomplished by the alcohol method of Blank and Tally (2). This method was used to avoid transformations which might occur during autoclaving. They were added aseptically to the basic medium which had been autoclaved.

Growth on xylose and dextrin was excellent; that on dextrose, levulose, galactose, mannose, cellobiose, maltose, sucrose, raffinose and xylan was good; that on arabinose and pectin was fair; while that on rhamnose, lactose, melibiose, cellulose, inulin, and starch was poor. Ethanol was utilized as well as sucrose at the lower concentration while glycerol, mannitol, malic acid and sodium succinate supported fair growth. Little or no growth occurred on methanol, propanol (iso), butanol, erythritol, dulcitol, phloridzin, calcium gluconate, or on tartaric, fumaric, succinic, glutaric, and tannic acids (Fig. 10).

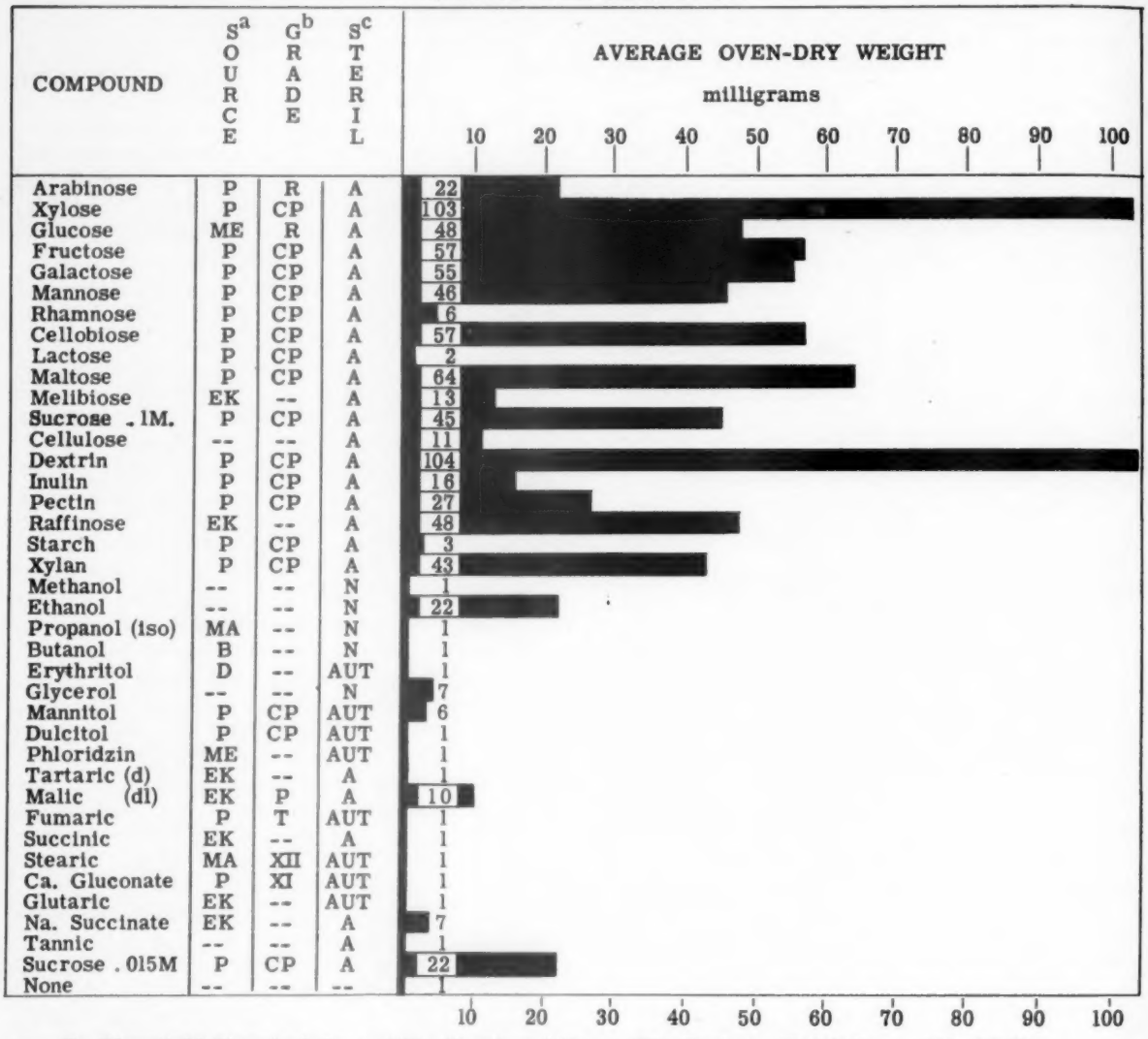
DISCUSSION.—The composition and formulation of special media depends upon their use. In metabolic studies only a minimum support of growth may be required. The physical-chemical conditions or nutrient components which best promote the production of one metabolite, as in a shunt metabolism, often differ from those which promote the efficient, balanced metabolism required for optimum growth (3, 4). Media to be used in isolation work, on the other hand, should combine good growth properties with selectivity. Here, however, the secondary organisms encountered would likely vary with the host species, the geographical region and, in the study of long distance spread, with the vector. The use of a single selective medium therefore has some obvious drawbacks.

Some differences in the best initial pH for growth which have been reported are apparent. White and Wolf (10) found best growth at approximately pH 4.0 and Hoffman (6) between 4.2 and 5.1. This level is similar to the 4.0 to 5.0 range reported here. Barnett and Lilly, however, reported that best growth occurred between pH 6.0 and 7.0 (1). Variation among isolates tested and nutrient sources supplied in the basal medium may account for this difference in part. A difference in the buffer level used might be responsible. In weakly buffered media a rapid drop in pH to 3.0 or below resulted in limited growth. A higher initial pH, though not optimum, would allow additional growth before this lower limit was reached. The use of 12 gm. of monopotassium phosphate per liter of medium adjusted and maintained the medium in the optimum range found in this study (approximately pH 4.5).

The benefit from vitamins for this isolate of *Chalara* was found to be slight. However, a high degree of deficiency for biotin has been reported (1). Since vitamin requirements among isolates may vary, their use may be advisable in metabolic studies. On the other hand, their use in selective media is questionable. The benefit to other microorganisms might be great also. This seems particularly true since at least 3 vitamins may be involved and since benefit from these 3 is so common among fungi.

Problems arise also in choosing the best nitrogen sources for specific uses. In differential media, the highest level of growth consistent with selectivity is desirable. The growth of *Chalara* was considerably greater with arginine than any other nitrogen source. Under some conditions this may outweigh any ad-

CARBON NUTRITION


COMPOUND
SOURCE[a]
GRADE[b]
STERIL[c]
AVERAGE OVEN-DRY WEIGHT
milligrams
10 20 30 40 50 60 70 80 90 100
Arabinose P R A 22
Xylose P CP A 103
Glucose ME R A 48
Fructose P CP A 57
Galactose P CP A 55
Mannose P CP A 46
Rhamnose P CP A 6
Cellobiose P CP A 57
Lactose P CP A 2
Maltose P CP A 64
Melibiose EK -- A 13
Sucrose .1M. P CP A 45
Cellulose -- -- A 11
Dextrin P CP A 104
Inulin P CP A 16
Pectin P CP A 27
Raffinose EK -- A 48
Starch P CP A 3
Xylan P CP A 43
Methanol -- -- N 1
Ethanol -- -- N 22
Propanol (iso) MA -- N 1
Butanol B -- N 1
Erythritol D -- AUT 1
Glycerol -- -- N 7
Mannitol P CP AUT 6
Dulcitol P CP AUT 1
Phloridzin ME -- AUT 1
Tartaric (d) EK -- A 1
Malic (dl) EK P A 10
Fumaric P T AUT 1
Succinic EK -- A 1
Stearic MA XII AUT 1
Ca. Gluconate P XI AUT 1
Glutaric EK -- AUT 1
Na. Succinate EK -- A 7
Tannic -- -- A 1
Sucrose .015M P CP A 22
None -- -- -- 1
10 20 30 40 50 60 70 80 90 100

a. P, Pfanstiehl Chemical Co.; ME, Merck and Co.; EK, Eastman Kodak Co.; MA, Mallinekrodt Chemical Co.; B, Baker Chemical Co.; D, du Pont.

b. R, Reagent grade; CP, Chemically pure; P, Practical; T, Technical; XII, U.S. Pharmacopoeia XII; XI, U.S. Pharmacopoeia XI.

c. A, Alcohol; N, no sterilization necessary; AUT, Autoclaved.

FIG. 11. The average oven-dry weights of mycelium produced on media containing various carbon sources.

vantage to other microorganisms afforded by organic nitrogen. In other circumstances, the use of ammonium nitrogen may give *Chalara* greater advantage over associated organisms to which this source is poorly or not at all available. Furthermore, if a specific shunt metabolism should be desired, differences in isoelectric point, mobility, effect upon O-R potential, etc. might alter the situation considerably. However, arginine was the best nitrogen source for general purposes.

Best growth was obtained at .018 molar concentration with 10 of the 16 nitrogen sources which supported growth. A higher concentration with 5 of the 6 remaining sources increased growth only slightly. Best growth with tryptophane was obtained at .006 molar concentration. Therefore, a concentration of approximately .02 molar nitrogen appeared suitable for most sources and for general purpose media.

The results obtained with carbon sources afford some basis for the development of differential media. Xylose and dextrin supported best growth. Many fungi use these to some extent. However, these sources were far superior to glucose which is perhaps the best carbon source for most fungi. On the other hand, for

general purposes, and for metabolic studies any of several mono- or disaccharides would be satisfactory.

The carbon-nitrogen ratio and total carbon in culture media is of considerable significance in metabolic studies (3, 4). As stated earlier, a .018 molar concentration of the best nitrogen sources supported a rate of growth comparable to higher levels. A .05 molar concentration of sucrose also supported a rate of growth comparable to higher levels when nitrogen was ample. Therefore, a medium containing 17 gm. sucrose and 3.5 gm. arginine per liter appears to be well balanced for mycelial growth.

The sparse growth of the fungus in wilting trees indicates that metabolites of high specific activity are operating in the disease situation. Enzymes and growth substances fit this qualification.

The development of tyloses in infected trees (9) indicates that dissolving or softening of structural barriers in the pit region has occurred. These barriers are composed largely of pectic materials. Defoliation also is prominent in the disease syndrome. Pectic enzymes normally are produced by deciduous plants at the time of abscission. These dissolve the pectic substances which cement the cells of the abscission layer (8). The possibility exists, therefore, that an abnormal production of these enzymes by the host is induced by the pathogen.

On the other hand, the utilization of pectin by *Chalara* in culture suggests that these may be produced by the pathogen itself. Several investigators have found toxic principles in media fermented by wilt fungi which exhibit properties similar to enzymes. Many other pathogens, such as the soft rot organisms, produce pectic enzymes. Brown (3) found that "the composition of the cultural medium affects the amount of enzyme secreted, so much so that in media with a high preponderance of carbohydrate over nitrogenous constituent very little enzyme is produced though there is considerable mycelial development. Conversely, in media with low carbohydrate and high nitrogen content growth may be poor but enzyme secretion is active." The concentration of carbon compounds in solution in the transpiration stream of a tree is probably low. Mycelial development is apparently poor within the vascular system of wilting oaks (11). However, enzyme production might be high under these conditions.

Growth substances such as heteroauxin also are commonly produced by fungi. In higher plants these are associated with the increased cell wall plasticity during cell elongation. The production of such a growth substance by *Chalara* could account for the abnormal growth of the ray cells in infected trees.

The working hypothesis appears that the oak wilt fungus produces pectic enzymes which weaken the structure of the bordered pits. Growth substances may increase the plasticity of cell walls. This permits the ray cells to grow into the vessels and to form tyloses. When such tyloses plug the vessels sufficiently, wilt develops.

SUMMARY

Chalara quercina grew best in liquid media buffered with 12 gm. of mono- and dipotassium phosphate proportioned to give a pH of 4.0 to 5.0. The addition of a minor element solution improved growth. A mixture of biotin, thiamine and inositol promoted growth. The deficiency for these appeared to be relatively slight, however.

Comparative yields on carbon sources were as follows: those on dextrin and xylose were best; on maltose, fructose, cellobiose, galactose, glucose, raffinose, mannose, sucrose, and xylan were good; on pectin, arabinose, inulin, and melibiose were fair; on rhamnose, starch, lactose and cellulose were poor. Among 9 alcohols and 9 organic acids, ethanol and malic acid supported fair growth at low concentrations.

Best nitrogen sources of 25 tested were arginine, asparagine and aspartic acid. Alanine, urea, methionine, and ammonium salts promoted fair growth. Phenylalanine, glycine, and histidine allowed sparse growth, while nitrate salts, isoleucine, threonine, hydroxypyroline, tryptophane, tyrosine, cystine, cysteine, lysine and leucine permitted very little or no growth.

Pectinase was produced in culture. This seemed important for the development of tyloses and subsequent wilt.

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HOST RESPONSES ASSOCIATED WITH THE DEVELOPMENT OF OAK WILT[1]

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The sudden wilting and death of oak trees soon after infection with *Chalara quercina* Henry (*Endoconidiophora fagacearum* Bretz) suggest a severe reaction of the host to the fungus. A study of the factors associated with these striking symptoms may clarify the mechanism of oak wilt development and may have implications for similar wilt diseases. Young (29) and Hoffman (13) have observed certain toxic effects on the leaves of oak cuttings placed in culture filtrates of this organism. However, the physiology of symptom development needs further study.

Reports concerning the wilting mechanisms in diseased plants have been reviewed by Brown (3), Harris (10), and Gottlieb (9). Water shortages from many causes may induce wilting. Root rot has been reported to produce such a water shortage (16, 18, 19). Plugging of the vascular system and a resulting water shortage have been attributed to masses of the pathogen (24, 25), to gums (2, 10, 17), to tyloses (8, 22, 30), to gas emboli (26) or to disintegration of the vessel walls into products which increased the viscosity of the tracheal fluids (21). Histological evidence and fluometric data supported these hypotheses. Increased transpiration also has been proposed (15), but needs clarification. Toxic metabolites produced by the incitant organisms have been considered important because filtrates of fermented media have produced toxic effects in cuttings. There has been considerable evidence to support this idea (7, 9, 30). However, toxin production has varied with the culture medium (20) and saprophytes as well as pathogens have produced such materials (1). In addition, Hodgson et al (12) showed that large molecules of many types could produce leaf flaccidity or injury. As a result, many toxic effects have been encountered which were probably foreign to the disease situation (3, 14, 21). Gottleib (9) obtained wilt with tracheal fluids from diseased tomato plants as well as with culture filtrates. Scheffer's findings suggest that even these may have contained products of a host-pathogen interaction rather than metabolites of the pathogen itself. In some instances, as in the Dutch elm disease, evidences both of plugging and of toxin effects have been found (30). In spite of differences in experimental approach, many investigators have concluded that disturbed water relationships were important for wilting.

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The term "toxin" generally has been used in the broadest possible sense, i.e., any metabolite of the pathogen which has a deleterious effect upon the host. Apparently the only restriction is that the harmful metabolite be liberated by the pathogen and move independently.

Except for occlusion by masses of the organism, any vascular plugging, tissue disintegration, change in cell permeability, or necrosis in the wilt syndrome might be associated with a "toxin". Considering the systemic nature of most wilt diseases, the great diversity of metabolites produced by microorganisms, the possible by-products of host-pathogen interactions, and the efficient transporting tissue into which these products may be liberated, a single effect upon the host would seem unlikely. The problem is to determine the abnormal effects induced in the host, their relative importance in bringing about the disease condition, and the nature of any active principles.

This paper reports changes in the water relationships which occur in oak wilt and discusses their significance.

MATERIALS AND METHODS. — Naturally occurring northern pin oaks (*Quercus ellipsoidales* Hill) in the central Wisconsin area were used throughout these studies.

Inoculum was prepared from transfers of a single-conidial isolate on potato-dextrose agar. Trees were inoculated through 2–3½ in. holes made in buttress roots. These holes were filled with 10 ml. of a heavy conidial suspension. Check trees received only water.

Radioactive rubidium^{86} carbonate was used in measuring the rate of movement of liquid in the transpiration streams of diseased and healthy trees. This readily soluble isotope has a 19½-day half-life. This is long enough to permit effective use and short enough to avoid disposal problems.

In the early experiments with this isotope little movement could be detected. A rate of movement of 1½ ft. per minute had been obtained with iodine^{131}, but this was less desirable than rubidium because of its expense and of its shorter half-life. Since potassium is chemically similar to rubidium, 1.5 per cent KCl was incorporated into the solution to reduce absorption of the isotope in the tissue. A rate comparable to that with radioactive iodine was then obtained.

Treatment with rubidium^{86} was made as follows. Asphalt paper cones were affixed to the trunks of trees 3–4 ft. above ground (Fig. 1A). Fibrated roofing cement closed the leaks. The KCl solution was added. Chisel cuts were made at least through the outer 2 annual rings of xylem. One-half a millicurie of rubidium in water solution was added. Radioactivity was detected with a portable, Geiger-type monitoring instrument (Nuclear Instrument and Chemical Company,

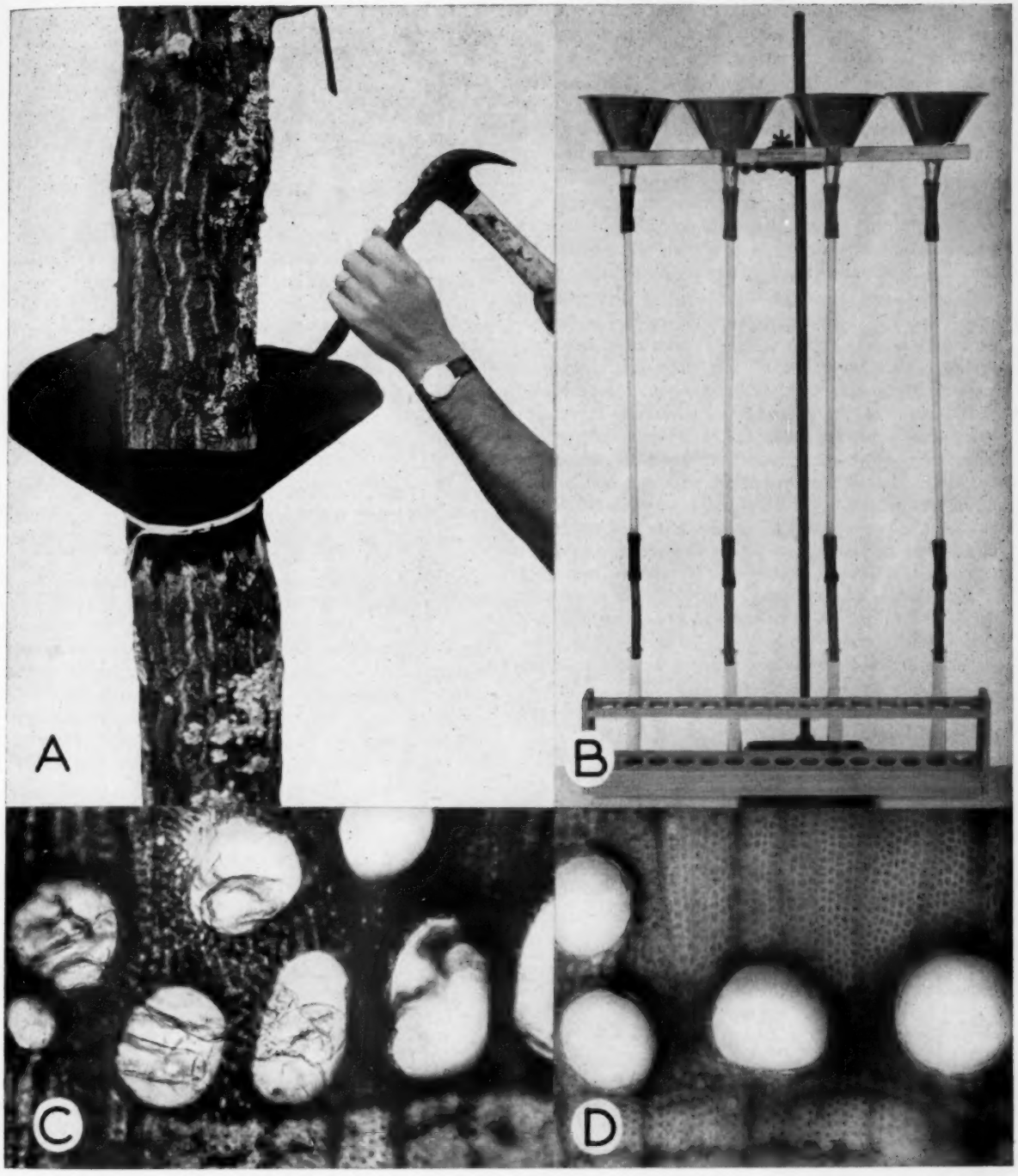

FIG. 1. A) A water-tight, asphalt paper cone fastened to the main stem of an oak tree. An aqueous solution of KCl and then of radioactive rubidium were introduced into the vascular system through chisel cuts into the wood. B) Apparatus for the measurement of water flow through 10 cm. twig cuttings with a pressure of 50 cm. of water. C) Cross section of the xylem of an inoculated northern pin oak showing tyloses in the vessels. D) Cross section of the xylem of an uninoculated northern pin oak showing open vessels.

Model 2611) with a usable sensitivity of approximately .05 milliroentgens per hour in the field. An activity twice that of the working background (.05-.07 mr. per hour) was set as a minimum for a positive reading.

Water flow through cuttings 10 cm. long and 8–10 mm. in diameter was used to determine the water-carrying capacity of diseased and healthy branches. These were placed under a 50 cm. column of water by

inserting one end into rubber tubing (Fig. 1B). A 10 cm. funnel at the top of each water column provided a reservoir to reduce variation in pressure. Ten such columns allowed the testing of many samples. The amount of water which passed through a cutting during 15 minutes was measured. The storage of twig samples in water from 0–5 hours had no apparent effect on flow.

Materials for histological examinations were immersed in the common formalin-acid-alcohol fixative immediately following water flow tests. Free-hand sections of this material were stained with safranin "o".

Experimental results.—The rate of movement of liquid upward in the transpiration streams of inoculated and noninoculated trees was measured by introducing radioactive rubidium into the vascular system. In 1951, 20 inoculated and 20 noninoculated trees 25–30 ft. high were treated as above. Rubidium movement was traced in healthy trees and in infected trees which showed no symptom or incipient, moderate, or severe wilt. This isotope moved approximately a foot a minute in the healthy trees. In general, a comparable rate was found in inoculated trees which showed no symptoms. In 2 symptomless trees, however, movement was extremely slow. Initial wilt symptoms appeared on these 2 trees 2 days later. Movement in trees with incipient and severe wilt symptoms was reduced by 90 and 99 per cent, respectively. These results demonstrated a rapid and severe drop in movement in the transpiration streams of infected trees. This drop was detected at the time of or slightly before initial wilt symptoms.

An attempt was made to discover the cause of this reduced movement in the transpiration stream. Water flow through twig sections from 20 inoculated and 20 noninoculated trees, 50–60 ft. high, was determined in July. Random samples were taken every fourth day through 16 days after inoculation. Thereafter, 10 trees were sampled every day through 34 days. Five branches were taken from various parts of each tree at each sampling. Generally, 2 10-cm. cuttings were obtained from a branch. The average water flow through twig sections of normal trees was approximately 4 ml. in 15 minutes. A gradual decrease to about 2 ml. occurred during the season. The flow for inoculated trees was similar until 3 days before wilt at which time there appeared to be a slight drop. By the time of incipient and severe wilt symptoms, however, the water flow was reduced by 80 and 95 per cent, respectively. These results indicated that vascular plugging was sufficient to account for the reduced movement in the transpiration streams. However, the time at which this plugging occurred was not yet closely defined.

A daily record of the flow of liquid in the transpiration stream and of the conductive capacity of the xylem, particularly during the period immediately preceding initial wilt symptoms, was sought in 1952. Sixty trees, 30–35 ft. high, were inoculated. Ten trees were treated with rubidium each day, beginning with the fourteenth day after inoculation, and were tested in numerical order without regard for the time of symptom development. All data were organized and computed according to the time interval between first wilt symptoms and the time of testing with rubidium. For example, data for all trees that wilted 5 days after testing were averaged.

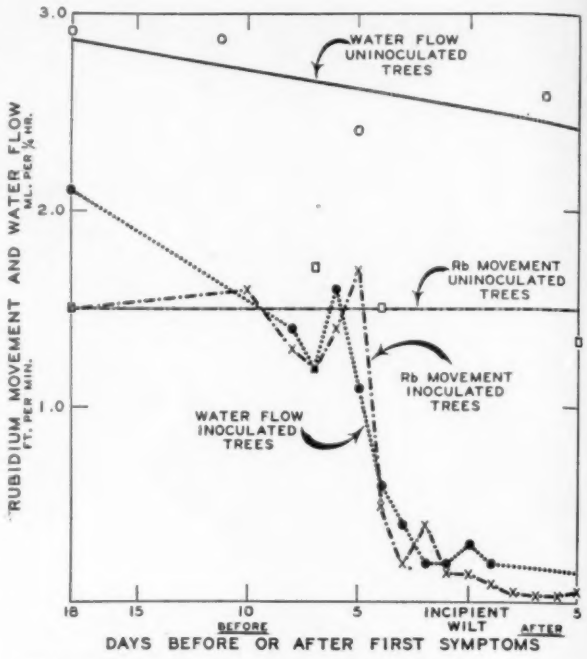

Fig. 2. Rubidium movement and water flow in inoculated and noninoculated northern pin oaks during July, 1952.

To study water flow, 20 trees with large crowns were selected in order to provide ample sampling material. Test samples were taken from 10 inoculated and 10 check trees. Daily samplings were made on each tree from the twelfth day following inoculation through severe wilt symptoms. Sampling and testing methods were similar to those in 1951. Flow data were compiled according to the time initial wilt symptoms appeared on each tree (Fig. 2). Each point in the graph represents an average from 80–100 readings.

A gradual drop in water flow through twig cuttings from check trees occurred during the course of the season. The average rate of rubidium movement in similar trees was relatively constant throughout the season and averaged 1½ ft. per minute. Normal water flow and rubidium movement within the normal range of variability were found in inoculated trees through the fifth day preceding initial wilt symptoms. This movement and the water carrying capacity dropped rapidly during the fourth and third day before permanent wilt until these had been reduced by 85–90 per cent. A further but gradual decrease occurred during the development of moderate and severe wilt symptoms. These results confirmed those obtained in 1951. They showed, in addition, that the occurrence of vascular plugging was correlated with the

reduced movement in the transpiration stream in time as well as extent.

During the period of low water conduction preceding permanent wilt, diurnal drooping and recovery of leaves were commonly observed in all these experiments. This indicated that the water supply had been reduced and was approaching the threshold for permanent wilt.

A histological examination of cutting material from trees with incipient or severe wilt symptoms showed that approximately 60 per cent of the vessels of the outer annual ring in any given focal plane contained tyloses (Fig. 1C). Since these tyloses were produced only where ray parenchyma cells were adjacent to vessels, it is likely that this 60 per cent represents the maximum occlusion possible in any given focal plane. It is likely, therefore, that all vessels were plugged at some point. On the other hand, tyloses were seldom observed in material from noninoculated trees (Fig. 1D). Details about this work have been reported by Struckmeyer et al (25).

The possibility that these tyloses may have been the result rather than the cause of the reduced movement in the transpiration stream was examined, since some investigators have proposed that they develop when conduction ceases. This work has been reviewed by Chattaway (4). To test this idea, 20 trees, 10–15 ft. high, were inoculated. The transpiration of these and noninoculated checks was severely reduced by clipping off all leaves. Five trees were defoliated at the time of inoculation and 5, respectively, at 1, 2, and 3 weeks thereafter. Other inoculated trees were not defoliated. Bud swelling, refoliation, symptom development, and water flow were recorded.

The vessels of all infected trees were severely plugged 3 weeks following inoculation, whether the trees had been defoliated or not (Fig. 3). However, no such tylosis formation and plugging occurred in noninoculated trees which had been defoliated. This constant association of tyloses with infection indicated that plugging was the response of the host to some action of the pathogen. Since plugging did not occur in defoliated check trees, it apparently was the cause rather than the result of the reduced movement in the transpiration stream.

Initial bud swelling in inoculated trees occurred 2–3 weeks after defoliation. Leaf development was complete in 5–6 weeks.

In a few inoculated trees no bud activity was apparent. Generally, however, bud swelling in these trees, as in the noninoculated trees, occurred 2–3 weeks following defoliation. Further development became increasingly retarded after bud break. The expanded buds withered at approximately the same stage of development whether the trees were defoliated at the time of inoculation or 3 weeks after inoculation when wilt symptoms were already apparent. No evidences of toxic action on leaves were observed.

Results indicated that leaves were not essential for disease development, and also that insufficient water

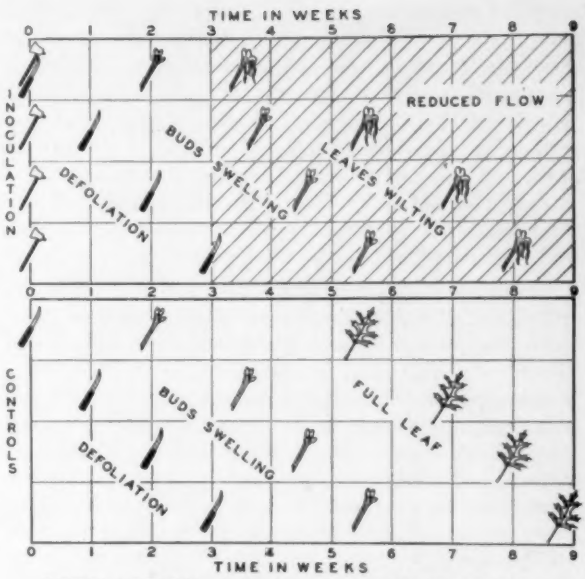

FIG. 3. Diagram of the design and of the results from the defoliation experiments. Inoculations were all made at 0 weeks. Defoliations of inoculated and check trees were made at 0, 1, 2 and 3 weeks. The time of plugging was indicated by cross-hatching. Bud swelling and wilting in inoculated trees were correlated with bud swelling and full leaf development in the check trees.

rather than a direct toxic action was responsible for leaf symptoms.

The ability of this vascular plugging to induce wilting was studied by interrupting the water-carrying capacity of 30 noninoculated trees mechanically. To do this, chisel cuts were made through 3–4 annual rings around 50, 75, 90 or 100 per cent of the circumferences of the main stems. In addition, the actual reduction accomplished by these treatments was measured on other trees as follows: Check trees were coned or felled and placed in containers of water. This provided a reservoir in which actual uptake of water could be measured. A 5-minute period was allowed for uptake to become stabilized. The volume of uptake during a 15-minute period was determined. The water carrying capacities of these trees were reduced by chisel cuts comparable to those above, i.e., around 50, 75, 90, or 100 per cent of the circumference. Again the uptake was measured for 15 minutes. In some instances rubidium^{86} was incorporated into the treatment so that a direct check could be obtained on movement into the crowns.

In trees which were girdled as much as 90 per cent of the circumference of the main stem, no leaf symptoms could be detected. When the outer annual rings were cut 100 per cent, however, diurnal drooping of the leaves with recovery at night commonly appeared. Drooping was most severe in the upper and outer portions of the crown. Permanent wilt set in 3–4 days later. Progressive bronzing of the leaves and defolia-

tion did not occur. In check trees similarly girdled 100 per cent, a 75–80 per cent reduction in water uptake was found at time of treatment. Thus the water supply in these trees was reduced to an extent comparable to that in pathological wilt. Under these conditions a sequence of diurnal drooping, permanent wilt, and death of these trees occurred, similar to that in pathological wilt. This provided added evidence that the water shortage found in trees infected with oak wilt could account for that wilt.

Discussion.—The rate of movement of radioactive rubidium in the transpiration streams of inoculated trees was normal through the fifth day preceding wilt. There was, therefore, no serious upset in the water conduction or transpiration by that time. A 90 per cent drop in movement in inoculated oaks occurred during the fourth and third days before initial, permanent wilt symptoms appeared. The drop in water flow through twig sections was almost identical in time and extent. These results showed that plugging of the vascular system and reduced movement in the transpiration stream preceded wilt symptoms, that they occurred simultaneously, and that plugging could account for the reduction in the transpiration stream.

A histological examination of the vascular tissue showed that tyloses were present to a considerable extent in diseased but not in healthy material. Some investigations, reviewed by Chattaway (4), have indicated that tyloses form when conduction ceases either as a result of injury or in the normal life of the tree. Mechanical defoliation reduced transpiration and movement in the transpiration stream. This defoliation failed to induce tylosis formation or plugging in noninoculated trees, however. On the other hand, tyloses did occur in defoliated trees which had been inoculated. This was correlated with inoculation and occurred regardless of the time of defoliation. Absence of tyloses or plugging when conduction was reduced mechanically, constant association of tyloses with infection, and the fact that plugging preceded any apparent leaf injury provided further evidence that tylosis formation was a response of the host to some action of the pathogen and that it was the cause rather than the result of the reduced transpirational movement.

The manner of defoliation is also significant. As stated earlier, initial bud swelling in inoculated trees occurred normally when compared to noninoculated trees. Further development, however, became more and more retarded. Eventually the small leaves withered and died at a late bud-break stage. This occurred regardless of the time interval between inoculation and defoliation (Fig. 3). It might be assumed from these results that some leaf development was necessary for the production of a toxic metabolite or that the leaves were not affected until adequate transpiration allowed such materials to accumulate in the leaves. Further consideration, however, invalidates these possibilities. Occasionally trees died before any bud activity was detected. In addition, plugging in defoliated as well as nondefoliated trees occurred 3 weeks following inoculation. In the first defoliation series no leaves were present during this entire period. In the final defoliation series leaves were present during the entire period and showed symptoms at time of defoliation. However, bud swelling, retardation of leaf development, and eventual wilting of the small leaves were comparable in both instances. These results indicated that development of the fungus and production of tylosis-inducing metabolites were independent of leaves.

A severe water shortage seemed to account for the results. Vascular plugging had reduced the water supply severely. In a few instances no bud swelling could occur. In general, however, buds developed normally to some extent. Because of the limited water supply further development then became more and more retarded. Eventually the increased demand for water exceeded the supply. First incipient and then permanent wilt developed.

The ability of this water shortage to induce wilt symptoms in infected oaks may be judged from results obtained when the water supply was interrupted mechanically. Diurnal drooping preceded permanent wilt in trees which were girdled 100 per cent. This suggests that the water supply was near the threshold for permanent wilt. A measure of the actual uptake of water before and immediately after this girdling showed that water availability had been reduced by 75–80 per cent. It is likely that some subsequent decrease occurred as a result of wounding during the 3–4 day period before permanent wilt. This reduction of water supply of somewhat more than 75–80 per cent required to produce wilt mechanically is similar to the 85–90 per cent reduction in water flow and rubidium movement found by the time wilt symptoms had developed on infected trees. It may be concluded, therefore, that the reduced water supply which results from a plugging of the vascular system in wilt-infected trees is capable of producing the wilting and death of these trees. In addition, diurnal drooping occurred first in the upper and outer portions of the crowns of these mechanically treated trees. Such a progression of symptoms is typical of oak wilt infected trees. This indicates further that a water shortage is a primary factor in causing the disease situation.

Factors other than water shortage seem to be involved in oak wilt. For example, defoliation did not occur in trees with cut vascular tissue. However, the structural barriers in the pit pairs which must be dissolved or softened before tyloses can form are related to those which are dissolved in the abscission layers at leaf fall. The mechanism which functions to induce tylosis formation may be correlated with that which induces abscission.

As stated earlier, metabolites produced by the fungus are probably involved in these mechanisms. Many workers (5, 20, 6, 9) have proposed that various materials including polysaccharides, which are products

of the pathogen in culture, may act as toxic agents in producing leaf symptoms. This does not appear to be the case in oak wilt. Symptoms which occurred before leaves became hardened in early summer showed no evidence of necrosis or other toxic action. These leaves drooped, shriveled, and died.

On the other hand, several investigators have reported toxic action with semi-purified filtrate preparations which exhibit chemical and physical characteristics common to enzymes (2, 28, 21). Sparse growth of the pathogen is common in wilt diseases including oak wilt. This sparse growth in itself indicates that metabolites of high activity are operating. Enzymes and growth stimulators are noteworthy in this respect. Brown (3) has reported significant results concerning the production of enzymes in culture. He states that, ". . . in media with low carbohydrate and high nitrogen content growth may be poor but enzyme secretion is active." The ratio of carbon to nitrogen in solution in the vessels may be relatively low. A metabolism which results in active secretion of enzymes or growth stimulators could account for the severe reaction induced by relatively sparse fungus growth. Furthermore, the nature of tylosis formation indicates that a softening or dissolving of structural barriers, a stimulation of growth, or both has occurred. Scheffer has proposed that a dissolving action on vessel walls occurs in tomato wilt (21). Epinasty and adventitious root development are common in this disease (27). Enzymes and growth stimulators might account for these observations. Of the proposed mechanisms for wilting, those of enzyme and growth stimulator action appear the most tenable for oak wilt.

A synthesis of such evidence suggests further research to test the following working hypothesis: The fungus produces peptic enzymes which weaken the bordered pits. Growth stimulators may increase the plasticity of cell walls. This enables the protoplasts of the ray cells to grow into the vessels and to form tyloses. When the vessels are sufficiently occluded, the sap stream is practically stopped and wilt results.

SUMMARY

A sudden drop in the rate of radioactive rubidium movement in the transpiration streams of inoculated oaks occurred during a period 3–4 days preceding first permanent wilt symptoms.

A drop in water flow through twig cuttings occurred, similar in time and extent to that of rubidium movement.

Alternate wilting during the day and recovery at night were commonly observed during the period of low water conductivity before permanent wilt set in.

Preliminary histological examinations showed that tyloses were present to a considerable extent in wilting but not in healthy material.

Artificial interruption of the transpiration stream by defoliation failed to induce tyloses in noninoculated trees. Tyloses were formed, however, in inoculated-defoliated trees. Results indicated that tylosis formation was a response of the host to some action of the pathogen and that tyloses were the cause rather than the result of the reduced transpiration stream. They indicated also that the development of the fungus and of any significant metabolites were independent of leaves.

Results following mechanical reduction of the water carrying capacity of the xylem indicated that a reduction comparable to that found in infected oaks was capable of inducing wilt and death of these trees.

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OBSERVATIONS ON RACES OF PHYTOPHTHORA INFESTANS IN MEXICO [1, 2]

W. R. Mills [3] and John S. Niederhauser [4]

Resistance to late blight in potatoes has been sought for over 100 years. Only during the past 25–30 years has the high resistance or immunity of several native Mexican species been recognized. At the present time, efforts to incorporate the resistance of these species, particularly *Solanum demissum*, into commercial varieties are being made in most of the important potato producing countries of the world.

When breeding with *S. demissum* was begun, only 1 race of *Phytophthora infestans* was known. As the work progressed, additional races appeared. Six races have been reported in the U. S. (4), 7 in Scotland (1), 8 in Germany (2, 3) and 9 in Holland (5). Although a large number of potato varieties, originating from *S. demissum* × *S. tuberosum* crosses, have been introduced as blight-resistant, the resistance has not long persisted. There is today no commercial variety of potatoes which will not blight.

Since *S. demissum* is native only to Mexico, where it grows abundantly in the highlands, it appeared very desirable to investigate the blight situation in that country. Until recently, almost nothing was known about the occurrence of blight in Mexico. The first report of the existence of *P. infestans* in Mexico was made by Reddick (6) who observed it on *Solanum antipoviczii*, in 1930. That *P. infestans* is, in fact, widespread in Mexico is proved by observations made by the junior author since 1948. In the potato production and breeding program conducted by him, potatoes have been planted in such widely separated areas as Guadalajara, Leon, Chihuahua, Saltillo, Toluca and the Valley of Mexico. In all cases, they blighted at once if grown during the rainy season and left unsprayed. Also significant is the fact that in several of these locations the recently named blight resistant varieties, such as Kennebec, Cherokee, Essex, etc., blighted just as quickly and severely as the "susceptible" varieties. This shows conclusively that at least 1 virulent race, in addition to the common race of other countries, is present in Mexico.

In addition to the positive evidence cited above, there are theoretical reasons for believing that additional races of *P. infestans* can be found in Mexico. Mills and Peterson (4) have shown that when the 3 major genes for blight resistance commonly present in *S. demissum* [5] (designated as genes B, C and D) become separated through crossing with susceptible varieties, virulent races of the fungus appear which are specific for plants carrying the particular gene. When the genes are recombined by hybridization, additional races of the fungus appear which are capable of attacking plants bearing gene combinations B and C, and B and D. No race has been found in the United States which would infect plants carrying both genes C and D, or the original *S. demissum* parents.

Individual collections of *S. demissum* are known which carry less than the full complement of genes for resistance (2). Such plants are susceptible to one or more known races. Other individuals, even though immune, if heterozygous, would segregate susceptible individuals. Since a heterogeneous population of *S. demissum* plants has undoubtedly been in close association with *P. infestans* for a very long period of time,

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[2] Work done in cooperation with the Secretaría de Agricultura y Ganadería, Mexico, D. F. and with the Departamento de Agricultura, Estado de Mexico, Toluca, Mexico. Authorized for publication on February 18, 1953, as paper No. 1788 in the journal series of the Pennsylvania Agricultural Experiment Station and as paper No. 170 of the Department of Botany.
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[5] A fourth gene recently noted by Black (1) and Mastenbroek (5) has not appeared in the *demissum-tuberosum* hybrids studied by Mills and Peterson.

the probability exists that many of the races which are capable of developing on *S. demissum* are already present in Mexico. Should such races prove to be present, then Mexico would obviously be the ideal location for further testing of potato selections immune to the known races.

The theory was put to test in the summer of 1952. A collection of differential varieties suitable for identification of the known races, and developed cooperatively between Pennsylvania State College and Cornell University, was brought to Mexico. In addition, there were included 134 selections, each carrying the 2 genes C and D, and immune to all races in the U.S. over a period of 1–3 years. The selections were planted in the Toluca Valley on July 1, 1952, in the center of a potato planting of about 12 acres. The susceptible variety Bintje was interplanted among the differential varieties and immune selections, and all were left unsprayed. Records of appearance and severity of blight were made at approximately weekly intervals.

On July 21, blight was first seen in the plot. At that time, 25 of the 30 Bintje check plants, 2 of the 5 differentials, and 8 of the previously immune "CD" selections exhibited 1 or more lesions. By July 29, only 10 "CD" selections were still blight-free. All differential and all check plants were infected. The 2 selections which were still blight-free on August 4 (Table 1) were small and slow in emerging and first showed infection on August 11.

As the blight progressed, it became evident that within the previously immune "CD" group there was a considerable range of resistance. For example, on August 4, 1 of the "CD" selections was dead with blight, whereas 19 selections were outstanding for their combined resistance and good vine characteristics. Resistance was expressed by comparatively few, slow-spreading lesions confined to the lower, older leaves. Young leaves showed necrotic flecks with an occasional small lesion which sporulated very slightly or not at all. But by August 25, it was observed that most of the "CD" selections which had previously appeared highly resistant were now less so. Large, sporulating lesions developed on all leaves and when the plot was harvested on September 19, only 2 varieties had as much as 20 per cent of their foliage remaining.

TABLE 1.—*Number of potato varieties, carrying genes C and D for late blight resistance, which showed various degrees of infection. Planted at Toluca, Mexico, July 1, 1952*

Degree of Infection	Aug. 4	Aug. 11	Aug. 20	Aug. 25	Sept. 2
none	2	0	0	0	0
1-5 lesions	19	1	0	0	0
slight	28	12	8	1	0
moderate	67	36	20	9	2
severe	17	67	75	65	30
dead	1	18	31	59	102

It has frequently been observed that resistance to blight in potatoes may decline as the foliage reaches senescene. Rainfall was excessive during the summer, averaging more than 20 in. per month during July and August. By the last of August, the water table was near the surface of the soil and the temperature was very cool. Terminal vine growth had ceased. It appears probable that the more severe attack on the resistant selections in late August was due to premature aging of the plants, accentuated by the poor growth conditions.

CONCLUSIONS AND SUMMARY

A group of potato selections, suitable for the identification of races of *P. infestans* known in the United States, plus 134 selections immune to those races, was planted in the Toluca Valley, Mexico, on July 1, 1952. All the immune selections carried genes C and D for resistance.

Within about a month after planting, all selections developed blight. The hypothesis presented above, that races of *P. infestans* capable of developing on *Solanum demissum* should be present in Mexico, is verified.

Resistance showed by several previously immune selections declined as those selections reached maturity. Physiological senescence was hastened by environmental conditions unfavorable to the crop.

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RESISTANCE OF SOLANUM SPECIES TO PHYTOPHTHORA INFESTANS IN MEXICO [1, 2]

John S. Niederhauser [3] and W. R. Mills [4]

The Toluca Valley lies about 30 miles West of Mexico City at an altitude of 8,400 ft. During the rainy season from May to September, the daily rains and cool nights provide an environment ideal for the development of potato late blight caused by *Phytophthora infestans*. This climate would be of no particular interest were it not for the fact that in the surrounding mountains and in the Valley itself there are a number of wild species of potatoes. Among these are *Solanum demissum*, *S. cardiophyllum*, and *S. bulbocastanum*, all of which have been noted in the literature for their high level of blight resistance or immunity. *S. demissum* has been used as the source of blight resistance in potato breeding programs all over the world.

Phytophthora infestans is perennially present in the Toluca area. It is probable that late blight has existed in this area for the many hundreds of years that the climate has been similar to what it is now and that wild species of Solanum have been present in the area. It is self-evident that resistant clones would have a better chance for survival in such an environment. Furthermore, the races of *P. infestans* found here might also be expected to have a level of virulence unequalled elsewhere in the world.

During the summer of 1952 the authors had the opportunity to observe the blight resistance of many of these wild species in their native environment in the Toluca Valley and nearby, and also in plantings in experimental plots and in controlled greenhouse inoculations. These observations and results are described in this paper.

LATE BLIGHT ON SOLANUM DEMISSUM.—On the slopes of the volcano Popocatepetl, a well-known station for *S. demissum*, several widely separated areas where this species abounds were observed from July through September. On the first visit, very few blighted plants were found among hundreds examined. These had only 1 or 2 lesions each. But by the middle of September, the great majority of *S. demissum* plants were infected, some severely blighted. Collections were made of blighted and blight-free plants.

From the blighted *S. demissum* plants, on Popocatepetl and elsewhere, a large number of isolations of *P. infestans* were made. At least 1 of these isolates is so virulent that it has produced blight lesions on every clone of *S. demissum* inoculated with it. Even clones on which no blight had been found in their native habitat have been susceptible when tested with this isolate in the greenhouse. Not all collections have been tested, but it is considered doubtful that any clone of *S. demissum* is resistant to all races which we now have in culture. It is questionable if blight immunity exists in *S. demissum*.

Blight susceptibility of individual clones of *S. demissum* is not new. But the apparent lack of immunity in any clone of this species suggests that potato breeders must revise the widely held concept of this species as a source of complete resistance to *P. infestans*.

Even though blight lesions can be commonly found on *S. demissum*, the plant is rarely killed by the attack. Indeed, in most cases the lesion tends to be slow in growth, with limited sporulation and with a marked halo. Often the attacked leaf turns yellow and drops off. This level of resistance would be extremely valuable in a commercial potato.

LATE BLIGHT ON SOLANUM CARDIOPHYLLUM.—Lesions of late blight have been seen on *S. cardiophyllum*. They are slow-growing, necrotic except for a very thin sporulating margin, and have a narrow, bright yellow halo. These lesions are uncommon, and no clone of *S. cardiophyllum* has been observed to be the subject of a severe attack.

One culture of *P. infestans* has been isolated from *S. cardiophyllum*. It grows slowly on lima-bean agar, and produces very few sporangia. Inoculations on completely susceptible potato varieties (such as Bintje) have failed. This failure was attributed to the absence of zoospores.

Unfortunately, *S. cardiophyllum* rarely sets fruit, and seeds are even more uncommon. Failure has met nearly all our attempts to cross it with other potato species, to make inter- and intra-clonal crosses, and to self individual plants. To date, the resistance of this species has not been available for wide use in breeding work.

LATE BLIGHT ON OTHER SPECIES OF SOLANUM.—*Solanum bulbocastanum*. No positive record of late blight has been made on this species by the authors. Suspicious lesions have failed to yield conidia or cultures. Fruits and seeds are rare, but a limited number of seed have been collected from 1 clone.

Solanum boreale. This species was susceptible in the field.

Solanum stoloniferum.[5] This species was susceptible

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[2] Work done in cooperation with the Secretaría de Agricultura y Ganadería, Mexico, D. F. and with the Departamento de Agricultura, Estado de Mexico, Toluca, Mexico. Authorized for publication on February 18, 1953, as paper No. 1789 in the journal series of the Pennsylvania Agricultural Experiment Station and as paper No. 171 of the Department of Botany.

[3] Plant Pathologist, Rockefeller Foundation, Mexico, D. F.

[4] Special Scientific Aide, Rockefeller Foundation, Mexico, D. F. (Associate Professor, Department of Botany, Pennsylvania State College, State College, Pa., on leave).

[5] *sensu* Correll, Donovan S. 1952. Section Tuberarium of the genus Solanum of North America and Central America. U. S. Dept. Agr. Monograph 11. 243 pp.

ble in the field.

Solanum pinnatisectum. No blight was seen on the 1 clone grown at Toluca in 1952. However, a blighted shoot was seen at Chapingo, in the nearby valley of Mexico. Nonetheless this species is considered to be very resistant.

Solanum jamesii. This species was susceptible in the field.

Solanum sambucinum (?). The identity of the clone grown at Toluca is in doubt, but has been tentatively named *S. sambucinum.* It was susceptible in the field.

Solanum iopetalum. This species was not tested at Toluca, but was susceptible at Chapingo.

DISCUSSION.—The finding of blight on most of the wild species of *Solanum* occurring in the region of the valley of Toluca has considerable significance to potato breeders interested in blight resistance. Most, if not all, wild species which have shown a high degree of resistance have come from Central Mexico.[6] Races of *P. infestans* which can attack these sources of resistance probably could attack any of the progeny evolved in a breeding program. Probably these virulent races will appear eventually wherever resistant varieties are developed; if this is true, the performance of the wild species in their native environment indicates the maximum level of resistance that can be hoped for from these sources.

There does exist in several of the wild species a high level of resistance that would be of great practical value if included in a commercial variety. In order to attain the goal of incorporating this high resistance in a commercial potato, the breeder necessarily must make his selections with the most virulent cultures available. It would be desirable to have the surviving material tested in the field in Mexico to determine its resistance when exposed to the collection of races that have evolved on the wild species there. A clone that exhibits a given level of resistance at Toluca can be experted to show at least this level of resistance elsewhere. During the summer of 1952, 134 clones were sent from the United States,[7] where they had been immune in several years of field trials; they represented the most resistant material yet developed in the United States. Artificial inoculations, using the most virulent cultures available to investigators there, had failed to produce infection. At Toluca, all these clones blighted severely, and only 1 showed enough resistance to give even a moderate yield. The problem of sorting out this resistant material may not be easy. If it can be done only by field testing, the physical difficulties posed for the breeder are enormous.

In the potato production and breeding program being conducted by the senior author in Mexico, European varieties with "partial resistance" are being used. Those varieties (such as Alpha, Gineke, Voran and Ostbote) can be satisfactorily protected by ordinary spray schedules, when more susceptible varieties cannot be produced commercially even with regular, thorough spraying. These European varieties showing this "partial resistance" are equally susceptible (or resistant) to all races, but have a certain tolerance that keeps unsprayed plots green for a longer time and makes it easier to control blight in the sprayed plots. This type of resistance, which some have attributed to "minor genes," is the only practical resistance at present available in commercial potato varieties to be grown in the high valleys of Mexico.

SUMMARY AND CONCLUSIONS

Solanum demissum, S. cardiophyllum, S. boreale, S. stoloniferum, S. pinnatisectum, S. jamesii, S. sambucinum, and *S. iopetalum* are all reported as blight susceptible at Toluca, Mexico.

S. bulbocastanum is the only species tested that cannot be said to be susceptible.

Though not immune, several clones of *S. demissum* and *S. cardiophyllum* showed a very high degree of resistance.

Some cultures of *Phytophthora infestans* isolated from *S. demissum* have been demonstrated to be pathogenic on all clones of *S. demissum* on which they have been tested.

If the high level of resistance present in some clones of *S. demissum* can be incorporated into a commercial potato, this resistance would be of practical value. The importance of even "partial resistance" has been demonstrated by several European varieties (Alpha, Gineke, Voran, Ostbote).

THE ROCKEFELLER FOUNDATION,
MEXICO, D. F.

[6] Reddick, D. 1939. Whence came Phytophthora infestans? Chron. Bot. 5: 410-412.

[7] Mills, W. R. and J. S. Niederhauser. 1953. Observations of races of Phytophthora infestans in Mexico. Phytopathology **43**: 454-455.

ELECTRON MICROSCOPY OF ELONGATED PARTICLES ASSOCIATED WITH WHEAT STREAK MOSAIC [1]

A. Herbert Gold, Byron R. Houston, and John W. Oswald

A wheat mosaic was reported from California in 1952 by Houston and Oswald (2) and was shown to be typical of the wheat streak mosaic described by McKinney (4). In electron micrographs, elongated particles have been found in wheat plants affected by this virus.

The material examined consisted of healthy Sonora wheat leaves, and leaves of plants that had been experimentally juice-inoculated with the California isolate of wheat streak mosaic virus. Samples were taken from diseased and healthy plants of the same age 10–60 days after inoculation.

Preparation for electron microscopy largely followed methods described by Backus and Williams (1) for virus assay. Weighed leaf samples were ground finely in a mortar with a little added water. Without further purification, more water was added to the homogenate making a dilution of 90 to 1, based on the fresh weight of the original tissue. To a 0.9 ml. aliquot of this diluted homogenate was added 0.1 ml. of Dow latex 580 G (lot 3584) which contained 1.6×10^{11} latex spheres per ml. The resulting mixture was sprayed onto collodion-coated specimen screens, with a spray gun capable of producing droplets having volumes of 10^{-9} ml. or less. Examination in the electron microscope was facilitated if the collodion-coated screens were pretreated before spraying by pouring over them a 0.05 per cent solution of serum albumin, draining, and drying.[2]

On examination in the electron microscope after shadow-casting the specimens with uranium, material from diseased wheat plants was observed to contain flexuous, elongated particles (Fig. 1) not present in preparations from leaves of healthy wheat plants. By use of the assay method of Backus and Williams (1), the number of particles per gram of diseased leaf tissue was estimated by counting the relative number of particles and latex balls in a series of droplet patterns.

It was thought of interest to compare the concentration of these particles with the concentration of tobacco mosaic virus (TMV) in an old infection on inoculated leaves grown under favorable conditions. The particle concentration was found to vary from 1×10^{11} particles to 5×10^{11} particles per gm. fresh weight. This is about 0.1 per cent to 0.5 per cent of the concentration of TMV particles in an old tobacco mosaic infection in Turkish tobacco (6). If one assumes the same specific gravity for these particles as for TMV particles, the weight yield of particles associated with wheat mosaic would be only 0.01 in 0.05 mg. per gm. fresh weight of wheat leaves. This compares with yields of 7 in 10 mg. per gm. of virus in a 15-day tobacco mosaic infection in Turkish tobacco (7). The difference is much greater than is encountered with fluctuations in TMV concentration due to ordinary changes in growing conditions.

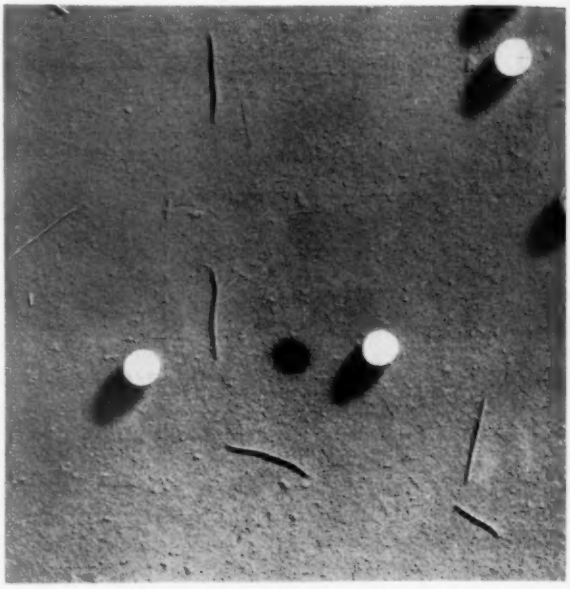

FIG. 1. Electron micrograph of elongate particles from wheat plants inoculated with California isolate of wheat streak mosaic, together with polystyrene spheres, 260 mμ in diameter, used for calibration.

On the basis of measurements of 100 particles in electron micrographs the average particle diameter was found to be 15 mμ, and the average length 635 mμ, with the most frequent length being 670 mμ (Fig. 2), based on calibration with polystyrene latex. The size range was much greater than that observed with TMV (8). The variation in size around 670 mμ appears to be too frequent and too great to be accounted for by addition to or breaking off from particles of a particular length. The evidence, therefore, appears to indicate that these particles actually vary in length but most of the particles are between 600 and 700 mμ in length.

Partly, perhaps, because of this very low ratio of supposed virus to normal cell constituents, it has not yet been possible to separate completely the elongated particles from host material. Some concentration of the particles has been achieved by alternating low speed centrifugation (10 minutes at 6,000 × gravity) and high speed centrifugation (1 hour at 110,000 × gravity), and by fractional precipitation with ammonium sulfate. The particles from wheat streak mosaic in preparations buffered at neutrality are not precipitated at 20 per cent saturation with ammonium sulfate, but are precipitated at 30 per cent saturation. Fractions from various combinations of these 2

[1] Accepted for publication May 1, 1953.

[2] Suggested by Dr. Robert C. Backus.

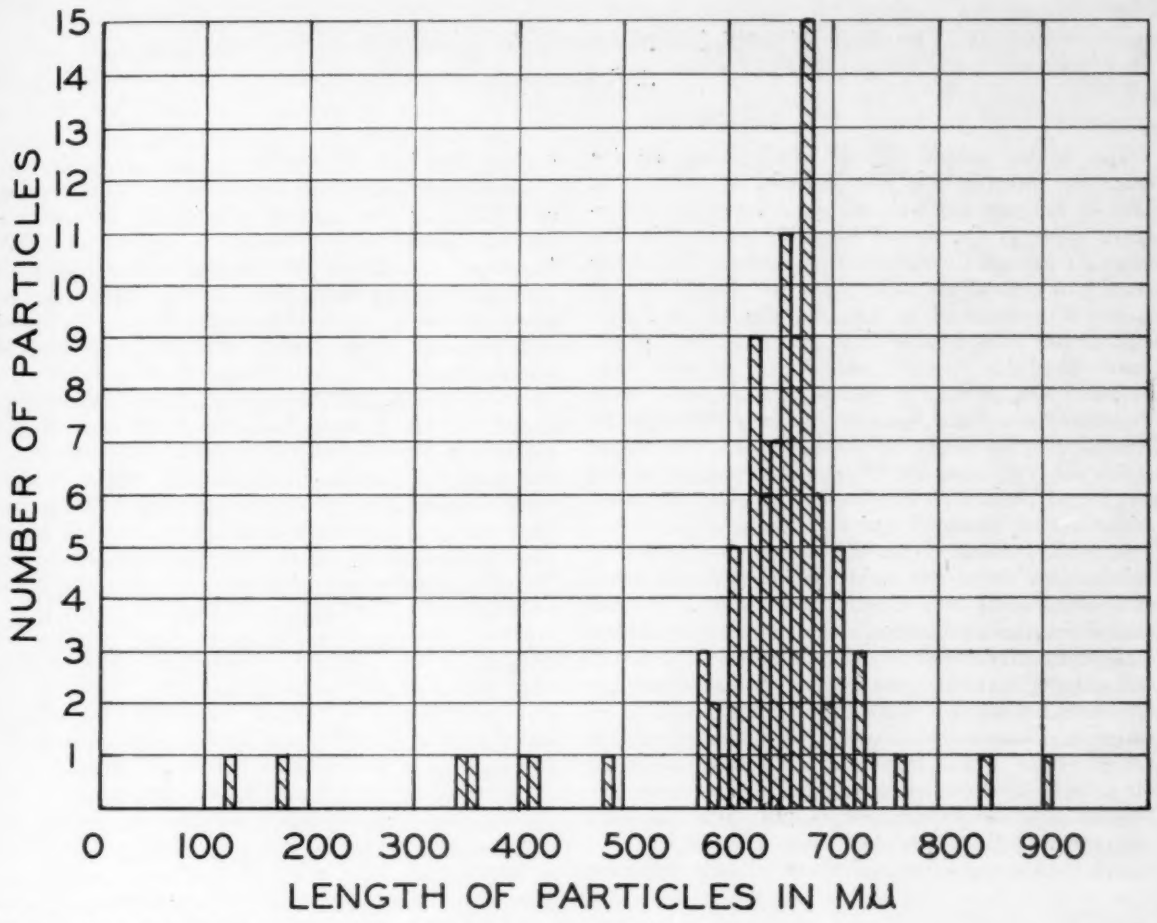

FIG. 2. Length distribution of particles from wheat mosaic plants.

treatments have been found to be infective when tested on wheat plants, if rods were present in the associated electron micrographs, but not otherwise. In no case to date has it been possible to produce a preparation which consisted entirely, or even predominantly, of the particles. For this reason, it is not possible to assert without reservation that the elongate particles observed constitute the infective agent.

SUMMARY

Flexuous elongated particles, 15 mμ in diameter, and with a most frequent length around 670 mμ were observed in Sonora wheat plants which had been artificially inoculated with the California isolate of wheat streak mosaic virus. These particles were absent in healthy plants. In old infections the particle concentration was about 5×10^{11} particles per gm. fresh weight of leaves. Efforts to separate these particles from other tissue components have not been successful to date, but fractions containing the particles were infective and those not containing them were not infectious.

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RACE 4 OF THE SOYBEAN DOWNY MILDEW FUNGUS [1]

S. G. Lehman [2]

The downy mildew disease of soybean, *Glycine max* (L.) Merrill, was first reported in America in 1924 by Lehman and Wolf who gave the name *Peronospora sojae* to the causal fungus.[3] Since then the name *Peronospora manshurica* (Naoumoff) Sedow has come into general use for this fungus. The disease it incites is widespread in America, Europe, and Asia and causes considerable damage to soybean plants under favorable weather conditions. In field comparisons, the amount of downy mildew on certain varieties has often appeared to vary differentially from locality to locality in a given year. This observation has suggested that *P. manshurica* comprises a number of physiologic races, some of which are present in certain localities and not in others.

In 1950, Geeseman[4] published results of experiments in which he tested the pathogenicity of 9 collections of *P. manshurica* on 7 varieties of soybeans. He concluded that the 9 collections included at least 3 physiologic races. He designated these as races 1, 2, and 3 and showed that they can be distinguished by their differential reaction to Richland and any 1 of 5 other varieties—Dunfield, Mukden, T 117, Manchu 3, or Chief. Race I was found in collections made at Madison, Wisconsin; Edgewood, Freeburg and Urbana, Illinois; Stoneville, Mississippi; Sikeston, Missouri; and Bella Mina, Alabama. Race 2 was obtained from a collection at Urbana, Illinois and race 3 from Nashville, Tennessee.

Greenhouse tests made by the writer in the spring of 1952 show that another physiologic race of the soybean downy mildew fungus is present in North Carolina. Conidia of the North Carolina race were obtained from 2 young, systemically infected Roanoke plants grown in the greenhouse from seeds encrusted with oospores. The quantity of conidia thus obtained was increased by inoculating and growing the fungus on other young Roanoke plants. Using conidia produced in this manner, inoculations were made on young plants of 20 varieties including 6 of the varieties used by Geeseman. Each plot consisted of 1 pot containing 5 plants of a single variety. Two tests were made, the differential varieties of test 1 being inoculated on April 12 and those of test 2 on May 26. The varieties were randomized in each of 4 replications in test 1 and of 2 replications in test 2.

Mildew developed rapidly in test 1 on varieties susceptible to the North Carolina race. Eleven days after inoculation the varieties of test 1 were rated for severity of disease on primary leaves using the severity rating system, 0, 1, 2, 3, and 4 published by Geeseman. Disease ratings for the 20 varieties are in Table 1.

The North Carolina race, here designated physiologic race 4, failed to infect any of the varieties used by Geeseman in differentiating races 1, 2, and 3. In his experiments races 1, 2, and 3 each produced severe disease on Illini, and severe or mild infection on Richland. Race 4, tested in North Carolina, induced no disease on either of these varieties. However, it did induce severe infection, rated as severity class 3 or 4 of Geeseman's scale, on Roanoke, Rokusun, Abura and N48-1867 (Roanoke × N45-745). Also, it caused mild infection, rated as severity class 1, on Acadian and Woods Yellow Sel. No infection by race 4 occurred on Dorman, CNS, Palmetto, Ogden, Rose Non-Pop, Ralsoy, N49-2370 (Ralsoy × Ogden), Chief, Manchu, Mukden, Dunfield, Richland, or Illini.

Results of the second test were like those of the

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[2] Professor of Plant Pathology, North Carolina State College, Raleigh, N. C.

Appreciation is expressed to Dr. C. R. Weber, Iowa State College and Dr. H. W. Johnson, North Carolina State College for supplying seed of the different soybean varieties.

[3] Lehman, S. G. and F. A. Wolf. 1924. A new downy mildew of soybeans. Jour. Elisha Mitchell Sci. Soc. 39: 164-169.

[4] Geeseman, G. E. 1950. Physiologic races of Peronospora manshurica on soybean. Agron. Jour. 42: 257-258.

TABLE 1. *Disease rating of 20 soybean varieties to physiologic race 4 of Pearonospora manshurica*

Variety	Reaction Test 1[a]	Test 2[b]	Variety	Reaction Test 1	Test 2
Chief	0	0	Woods Yel Sel.	1	0
Manchu	0	0	Acadian	1	0
Mukden	0	0	Dorman	0	0
Dunfield	0	0	CNS	0	0
Richland	0	0	Palmetto	0	0
Illini	0	0	Ogden	0	0
Roanoke	4	1,2	Rose Non Pop	0	0
Rokusun	3,4	1,2,3,4	Ralsoy	0	0
Abura	3,4	1,2	N49-2370	0	0
N48-1867	4	0,1,2	N49-2560	–	1,2,3

[a] Inoculated April 12
[b] Inoculated May 26

first, except that infection ratings of the susceptible varieties were uniformly lower in the second test; Woods Yellow Sel. and Acadian, mildly affected in the first test, showed no symptoms of mildew in the second. This difference was due, it is believed, to inability to maintain temperatures low enough for abundant production of conidial inoculum in the second test. N49-2560 (S100 × CNS), used only in the second test, reacted much like Roanoke and Rokusun.

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PHYTOPATHOLOGICAL NOTES

Compatibility of Acti-dione and Orthocide in the Treatment of Seed.[1] A. W. HENRY, E. A. PETERSON AND R. L. MILLAR. Good control of covered smut of wheat has been achieved with the antibiotic, Acti-dione, by applying it to infested seed in .5 and 1 per cent dust mixtures with Dixie clay at ½ oz. per bushel.[2] Control has been effected without significant seed injury but also without significant improvement as measured by seedling emergence.

If Acti-dione should prove compatible with certain other fungicides it seemed possible that it might be used in a combination treatment to control covered smut and improve emergence. In preliminary trials in the field and in the greenhouse at the University of Alberta, a limited number of combinations have been tested. One of the most promising has been that of Acti-dione and Orthocide.[3] Acti-dione (cycloheximide) was used in mixtures with Dixie clay while commercial dust preparations of Orthocide 406 (containing N-trichloromethylthio tetrahydrophthalimide as the active constituent) were employed. Rates as low as ½ oz. per bushel of each have given promising results. Acti-dione alone has not improved emergence over that of the untreated check and Orthocide alone has not in our tests controlled covered smut of wheat satisfactorily but when certain wheat samples treated with both have been sown, covered smut has been controlled and emergence has been improved.—Division of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.

[1] Financial assistance from the National Research Council of Canada is gratefully acknowledged.

[2] Henry, A. W., R. L. Millar, and E. A. Peterson. 1952. Control of covered smut of wheat by rapid seed treatment with an antibiotic. Science 115: 90-91.

[3] Kindly supplied by the Upjohn Company of Kalamazoo, Michigan, and the California Spray-Chemical Corporation of Richmond, California, respectively.

Association of Ergot with Loose Smut of Wheat and of Barley.[1] W. J. CHEREWICK. In the study of physiologic specialization in loose smut of wheat and of barley (*Ustilago tritici* (Pers.) Rostr. and *U. nuda* (Jens.) Rostr.) collections from various parts of Canada were subjected to tests on a series of differential host varieties. Frequently infections of ergot (*Claviceps purpurea* (Fr.) Tul.) have been observed in field plots of these cereals inoculated with loose smut collections. During the winter of 1950-51 several collections of loose smut made in 1950 produced ergot infections following inoculations of wheat and barley plants in the greenhouse, and in the following winter over 50 per cent of loose smut collections made in 1951 produced ergot infections. Results of these inoculation experiments suggest that the higher proportion of loose smut collections found to be contaminated by ergot in 1951 reflects a greater prevalence of ergot in cereals in that year than in 1950.

Microscopic examination of a number of loose smut collections that produced ergot infections upon inoculation, revealed an abundance of ergot conidia among the smut spores. One of these contaminated smut collections, held 18 months at ordinary room temperature, contained viable ergot conidia that caused infections in floral inoculations, whereas conidia in dried-up honey-dew, stored the same length of time under the same conditions, failed to produce any infection. It appears, therefore, that smut spores provide shelter for and probably maintain the moisture content of the rather delicate conidia of ergot.

To elucidate the possible ways by which ergot conidia become associated with loose smut spores in nature, a number of tests were made in the greenhouse, to determine whether or not ergot can develop on plant tissue other than young ovaries. Inoculations of barley and wheat florets with ergot conidia following removal of the ovaries and stamens before anthesis usually resulted in the development of the sphacelial stage, particularly when inoculated plants were kept in a humid atmosphere. In some cases, irregular sclerotia developed in these empty glumes. Inoculations of plants with ergot, before the emergence of heads, by injections of a conidial suspension into the sheaths, resulted in transformation of the entire head into an ergot sclerotium (Fig. 1, A); frequently sclerotial masses developed in the stems between the upper node and the head (Fig. 1, C, left). When heads were cut off before inoculation, ergot sclerotia developed in the stem (fig. 1, C, right). Inoculations of smutted heads just before emergence resulted in the develop-

[1] Contribution No. 1272 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

FIG. 1. Illustrations of ergot infections on barley plants. A) Two heads inoculated before emergence and transformed into ergot sclerotia; B) sphacelial development on smutted spike; C) ergot sclerotia developed on young stems.

ment of the spacelial stage of ergot on parts of glumes that were not entirely destroyed by the smut (Fig. 1, B).

These inoculation tests indicate that ergot may develop not only upon young ovaries but on any physiologically young tissue of wheat and barley plants. The development of ergot on smutted heads of wheat and barley in nature would explain the abundance of conidia found in some loose smut collections. Moreover, the association of ergot conidia with loose smut spores that are naturally disseminated by air currents not only provides one method of ergot dissemination, but may

have some relationship to the high prevalence of ergot in loose smut susceptible varieties such as Montcalm barley and Lee wheat.—Laboratory of Plant Pathology, Winnipeg, Man., Canada.

Studies on the Control of Potato Late Blight (Phytophthora infestans) with Antibiotics[1]. REINER BONDE. Studies were conducted to determine whether solutions of certain antibiotics will prevent infection of potato

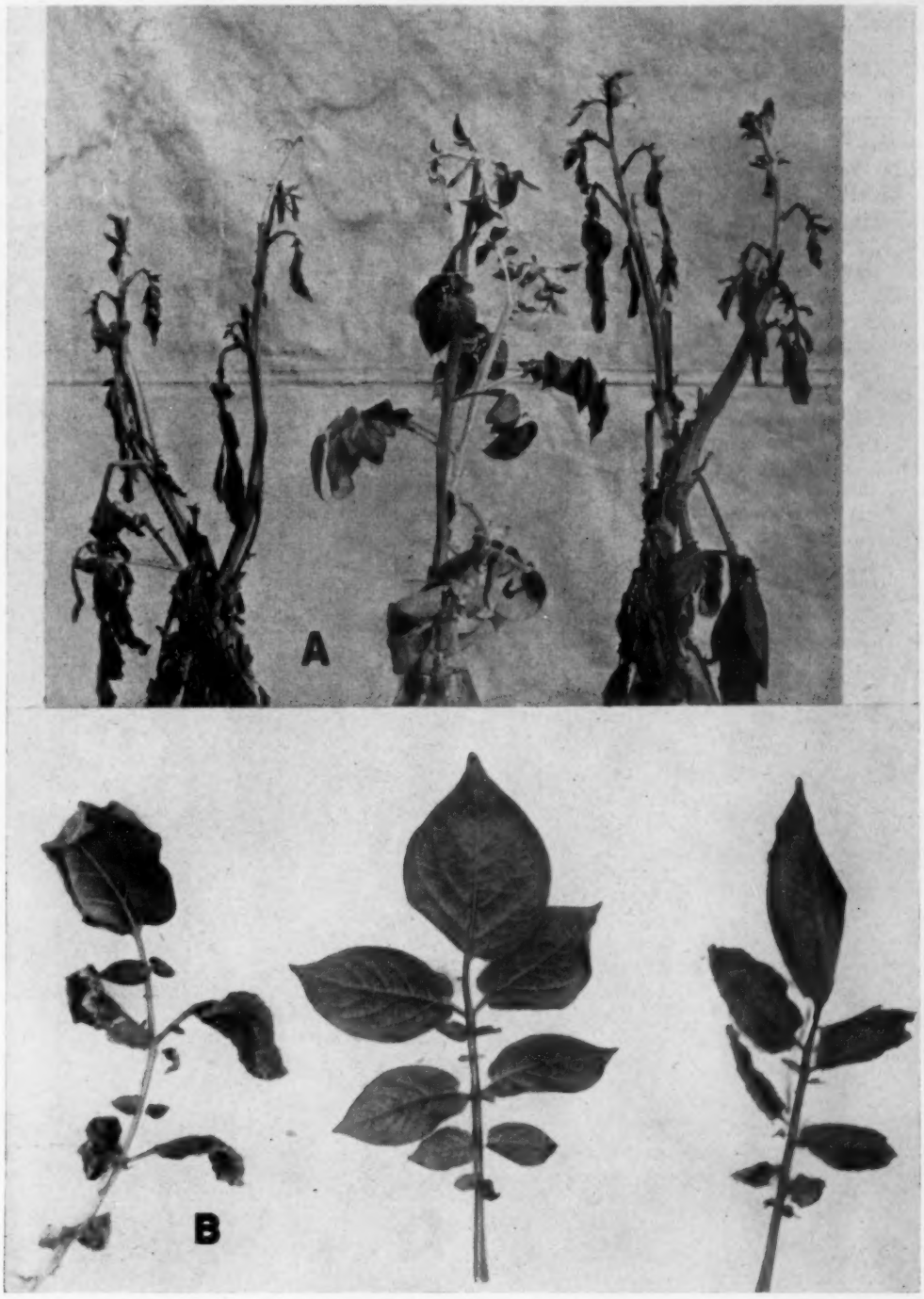

FIG. 1. Protection of potato against *Phytophthora infestans* by absorption of an antibiotic solution through cut stem for 72 hours before inoculation with conidia. A) Left to right, cuttings treated with thiolutin, terramycin hydrochloride, and sodium rimocidin, respectively. Note absence of lesions on stems of middle set of shoots. B) Left to right, leaves from shoots treated with sodium rimocidin, terramycin hydrochloride, and thiolutin, respectively. Note that the only symptoms on middle leaf are slight marginal necrosis and pinpoint lesions on blades.

plants by the late-blight fungus *Phytophthora infestans* (Mont.) DBy.

Young Katahdin potato plants were cut off at ground level and the cut ends immersed in solutions of thiolutin, sodium rimocidin, streptomycin sulfate, and terramycin hydrochloride, each containing 100 μg. per ml. After 6- and 72-hour periods in the solutions, the treated potato shoots and untreated controls were sprayed with an aqueous suspension of conidia and placed in a moist chamber.

None of the antibiotics reduced the amount of infection when the stems had been in the solutions for 6 hours. Terramycin hydrochloride, however, had a definite effect when the stems had been in the solution for 72 hours. The lesions were slower to appear and develop and remained as pin-point spots without rapidly killing the plants (Fig. 1). The infection was confined to the leaflets and did not cause lesions on the stems as was the case for the other antibiotics and the controls. The production of conidia on the leaves of plants treated with terramycin hydrochloride was absent or scanty whereas profuse sporulation occurred with the other antibiotics. Sodium rimocidin appeared to stimulate the formation of conidia. The stems treated with terramycin hydrochloride were about 25 per cent defoliated 8 days after inoculation, compared with nearly 100 per cent for the other solutions.

Potato plants were sprayed with the 4 solutions just before inoculation with a conidial suspension. In 1 experiment a spray application of freshly mixed thiolutin prevented infection.

Young potted potato plants were watered 3 times at weekly intervals with aqueous solutions or suspensions of 13 antibiotics (100 μg. per ml.) and then inoculated with conidia. Antibiotics included in the experiment were crude aureomycin, bacitracin, chloromycetin, dihydrostreptomycin sulfate, potassium penicillin, sodium penicillin G, polymyxin, rimocidin, sodium rimocidin, rimocidin sulfate, streptomycin sulfate, thiolutin crystals, and crude thiolutin. None of the antibiotics when applied to the soil prevented or reduced infection.

The antibiotics did not appear to be toxic to the potato when absorbed into cut stems, sprayed on the foliage, or applied to the soil.—Maine Agricultural Experiment Station, Orono, Maine.

[1] Project sponsored by the Chas. Pfizer Company, Inc., Brooklyn, N. Y.